June 2025

Investigation of Antibacterial and Antibiofilm activity of *Tinospora cordifolia* leaf extract

Project report submitted to Sister Nivedita University in partial fulfillment of the requirement for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

Submitted by

JYOTI PANDEY

REGISTRATION No.: 230020023192

Enrollment ID: 2322210002002

SESSION: 2023-2025

Under the guidance of

Dr. Moupriya nag and Dr . Dibiyajit lahiri,

Associate Professors, University of Engineering & Management (UEM)

School of Health Science and Translational Research

Sister Nivedita University

UNIVERSITY
OF ENGINEERING AND MANAGEMENT
(Established by Act XXV of 2014 of Govt. of West Bengal & recognized by UGC, Ministry of HRD, Govt. of India)
University Area, Plot No. III-B/5, Main Arterial Road, New Town, Action Area – III, Kolkata - 700160, WB, India
Admission Office: 'ASHRAM', GN-34/2, Salt Lake Electronics Complex, Kolkata - 700091, WB, India

Ph.(Office) : 913323572969
: 913323577649
Admissions : 913323572059
Fax : 913323578302
E-mail : vc@uem.edu.in
Website : www.uem.edu.in

CERTIFICATE

This is to certify that Mrs Jyoti Pandey, student of the Biotechnology Department, Sister Nivedita University, has successfully completed her dissertation entitled: "Investigation of Antimicrobial and Antibiofilm Activity of *Tinospora cordifolia* Leaf Extract" The dissertation was carried out under the mentorship of Dr. Moupriya Nag and Dr. Dibyajit Lahiri, Department of Biotechnology, University of Engineering and Management (UEM), Kolkata.

Date: _____

Moupriya Nag.

Dr. MoupriyaNag
Associate Professor
Department of Biotechnology
UEM, Kolkata

Dibyajit Lahiri

Dr. Dibyajit Lahiri
Associate Professor
Department of Biotechnology
UEM, Kolkata

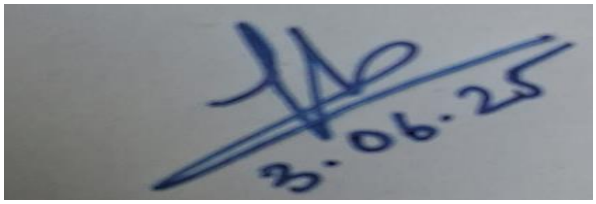
Other Institutes of the Group
University of Engineering & Management (UEM) Jaipur – 6 Km, from Chomu on Sikar Road (NH-11), Jaipur-303807, Rajasthan Ph. 01423-516102
Institute of Engineering & Management (IEM) – Salt Lake Electronics Complex, Sector-V. Kolkata- 700091, West Bengal Ph. (033) 2357-2969
IEM Public School – GE, 4/A, Sector-III, Salt Lake, Kolkata – 700106, West Bengal (Near Tank No. 12, Behind NIFT Girls' Hostel)

DECLARATION BY THE STUDENT

I hereby declare that the work which is being presented in this dissertation entitled "Investigation of Antibacterial and Antibiofilm activity of *Tinospora cordifolia* leaf extract" in partial fulfilment of the requirements for the award of the degree of Bachelors/Masters/Ph.D of--- Science in Biotechnology is an authentic rectiord of my own work carried out during the period from 17th February 2025 to 19th May 2025 under the supervision of Dr. Moupriya Nag and Dr.Dibyajit Lahiri Department of Biotechnology , University of Engineering (UEM). I acknowledge that I have read and understood the UGC regulations, 2018 (Promotion of Academic integrity and Prevention of Plagiarism in Higher Educational Institutions).

I confirm that this dissertation has been checked for plagiarism using the plagiarism checking software turnitin. At the end of the dissertation, a copy of the summary report demonstrating similarities in content and its potential source (if any), generated online using plagiarism checking software is enclosed. I herewith confirm that the dissertation has been less than 10% similarity according to the plagiarism checking software's report and meets the UGC Regulations as well as plagiarism rules of Sister Nivedita University.

I further declare that no portion of the dissertation or its data will be published without the Institute's or supervisor's permission. I have not previously applied for any other degree or award using the topics and findings described in my dissertations.



(Signature of the students with Date)

Name of the Student:- Jyoti Pandey

Registration no.:- 230020023192

Department :- Biotechnology

SISTER NIVEDITA UNIVERSITY
সিস্টার নিবেদিতা বিশ্ববিদ্যালয়

DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

TO WHOM IT MAY CONCERN

This is to certify that the project report titled " Investigation of antimicrobial and antibiofilm activity of Tinospora cordifolia", submitted by Mrs. Jyoti Pandey (Registration No. 230020023192, Enrolment No. 2322210002002) to the Department of Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfillment of the requirements for the Master of Science degree.

Date: 03.06.2025

Place: SISTER NIVEDITA UNIVERSITY

Signature of Head of the Department

Seal

Head
Department of Biotechnology
Sister Nivedita University
Block DG-1/2, Action Area 1
New Town, Kolkata-700 156

ACKNOWLEDGEMENT

I am grateful to my Vice chancellor Prof. (Dr.) Dhrubajyoti Chattopadhyay and Dr. Rajat Pal, Assistant Director - Microbiology and Biotechnology, SNU for his continuous guidance, encouragement, and support throughout my academic tenure.

I am especially grateful to Dr. Somsubhra Thakur Choudhury, Head of the Department of Biotechnology and Dr. Sudipta Saha, Associate Professor - Microbiology & Biotechnology, for their valuable insights and consistent motivation that played a crucial role in the successful completion of my work. Also allowing me to pursue my research work from UEM as part of the MSc Degree programme.

I would like to express my deepest gratitude to Dr. Sajal Dasgupta, Vice Chancellor, UEM, Kolkata for providing the technical and laboratory support to me.

I would like to express my deepest gratitude and sincere thanks to Dr. Dibyajit Lahiri & Dr. Moupriya Nag for providing this opportunity to do this research project in their laboratory and providing me an opportunity to work under their guidance. Their kind attention, care & continuous encouragement has been a source of inspiration for me throughout the project. It is only with their mentoring, leadership and cooperation that I was able to finish this thesis. I am highly indebted to them for sparing their valuable time for teaching me all the techniques. I am obliged and thankful to them for providing me the opportunity to gain knowledge and understanding of working skills of the aspects of my work.

I find this an appropriate chance to thank Prof. Sumita Mukherjee, HOD Department of Biotechnology for providing us continuous encouragement and support which has been a source of inspiration for us throughout the project. We are also grateful and offer our sincere thanks and gratitude to her.

I would also like to acknowledge the mentorship of Dr. Rupsa Bhattacharya, Associate Professor and Postdoctoral Researcher for her unwavering support, expert advice, and kind supervision which has been instrumental in shaping my MSc. research project.

General abbreviations:

Abbreviation	Full form
AAE	Ascorbic Acid Equivalent
ATCC	American Type Culture Collection
BSI	Botanical Survey of India
CD	Critical Difference
CFU	Colony Forming Units
CLSM	Confocal Laser Scanning Electron Microscope
Conc.	Concentration
EPS	Exopolysaccharide
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalent
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High-Performance Liquid Chromatography
IC_{50}	Half Maximal Inhibitory Concentration
KU	Kalyani University
L.	Linnaeus
MBC	Minimum Bactericidal Concentration
MCC	Microbial Culture Collection
MIC	Minimum Inhibitory Concentration
MTCC	Microbial Type Culture Collection
NICED	National Institute of Cholera and Enteric Diseases
NIST	National Institute of Standards and Technology
NO	Nitric Oxide
PCR	Polymerase Chain Reaction
QE	Quercetin Equivalent
QS	Quorum Sensing
qRT-PCR	Quantitative Real-Time PCR
RCF	Relative Centrifugal Force

Abbreviation	Full form
Rf	Retention Factors
Rpm	Revolutions Per Minute
RT	Retention Time
SEM	Scanning Electron Microscope
SO	Super Oxide
TAA	Total Antioxidant Activity
TFC	Total Flavonoid Content
TLC	Thin Layer Chromatography
TPC	Total Phenolic Content
TTC	Total Tannin Content
UK	United Kingdom
US	United States
UV-Vis	Ultraviolet-Visible
v/v	Volume/ Volume
w/v	Weight/ Volume

Chemical abbreviations:

Abbreviation	Full form
ABTS	2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
AHL	Acyl Homoserine Lactone
$AlCl_3$	Aluminum chloride
Amp	Ampicillin
BSTFA	N, O-bis (trimethylsilyl) trifluoro acetamide
cDNA	Complementary
$CuSO_4$	Copper sulfate
dd H_2O	Double distilled water
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTPs	Deoxy-nucleoside triphosphates

Abbreviation	Full form
DPPH	2,2-diphenyl-1-picrylhydrazyl
ECR	Elastin-congo red
EDTA	Ethylenediaminetetraacetic acid
EtBr	Ethidium bromide
FC reagent	Folin–Ciocalteu reagent
$FeCl_3$	Ferric chloride
Gen	Gentamycin
gDNA	Genomic DNA
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric acid
HCl	Hydrochloric acid
ITA	Itaconic anhydride
$K_3[Fe\ (CN)]_6$	Potassium ferricyanide
KI	Potassium iodide
LA	Luria Bertani agar
LB	Luria Bertani broth
$MgCl_2$	Magnesium chloride
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
NA	Nutrient agar
Na_2CO_3	Sodium carbonate
Na_2SO_4	Sodium sulfate
NaNO2	Sodium nitrite
NaOH	Sodium hydroxide
NaOH	Sodium hydroxide
NB	Nutrient broth
NBT	Nitro blue tetrazolium chloride
NPN	N-phenyl-1-naphthylamine
PBS	Phosphate buffer solution

Abbreviation	Full form
PI	Propidium iodide
RNA	Ribonucleic acid
SNP	Sodium nitroprusside
TAE	Tris-acetate-EDTA
TMCS	Trimethylchlorosilane
TPTZ	2,4,6-Tris(2-pyridyl)-s-triazine
Tris-HCL	Trisaminomethane-hydrochloric acid
TSA	Tryptic soy agar
TSB	Tryptic soy broth

Unit abbreviations:

Unit system / Symbol	Represents
%	Percentage
:	Ratio
A.U.	Arbitrary Units
°C	Degree Celsius
C_t value	Cycle threshold
°E	Degree East
°N	Degree North
µg	Microgram
µl	Micro litre
µS	Micro Siemens
Unit system / Symbol	**Represents**
Cm	Centimetre
G	Gram
H	Hour
L	Litre
M	Meter
M	Molar

Mg	Milligram
Min	Minute
Ml	Millilitre
Mm	Millimeter
mM	Millimolar
mV	Millivolt
N	Normal
Nm	Nanometer
nM	Nanomole
OD	Optical density
Sec	Second
U	Unit
v/v	volume/ volume
w/v	weight/ volume

INDEX

Section Title	Subsections	Page Number
Abstract	1.1 Overview of *Tinospora cordifolia* and its therapeutic significance	12
	1.2 Objectives and scope of the study	12
	1.3 Key findings and conclusions	12
	1.4 Keywords	12
Introduction	2.1 Botanical description and classification	13
	2.2 Distribution and habitat	13
	2.3 Historical and traditional significance	14
	2.4 Phytochemical profile overview	15
	2.5 Global research contributions and significance	15
Phytochemical Profile of *Tinospora cordifolia*	3.1 Major phytochemical classes in different plant parts	15
	3.2 Specific compounds identified in leaves, stem, roots, and whole plant	16
	3.3 Summary table of phytochemicals and references	18
Literature review on Antimicrobial Activity of *Tinospora cordifolia*	4.1 Bioactive compounds responsible for antimicrobial effects	19
	4.2 Mechanisms of antimicrobial action	21
Methodology	5.1 Extraction procedures	23
	5.2 Quantitative analysis (Phenolic content, etc.)	25
	5.3 Antimicrobial testing methods (Disc diffusion, MIC, etc.)	26
	5.4 Biofilm inhibition assays	27
Results and Discussion	6.1 Extraction yield and phytochemical analysis	31
	6.2 Antimicrobial activity findings (Disc diffusion and MIC.)	32
	6.3 Biofilm inhibition results	34
	6.4 Significance and implications of findings	35
Conclusion	7.1 Summary of key results	35
	7.2 Potential applications and future scope	37
	7.3 Relevance to traditional medicine and antimicrobial strategies	38
References		39

ABSTRACT

Tinospora cordifolia, commonly known as Guduchi or Giloy, is a traditional ayurvedic medicinal plant known for its broad-spectrum therapeutic properties . Every part of this plant is treasure of bioactive compounds , which has led to world wide research across 45 countries globally to explore and exploit its bioactive compound to its maximum potential. This study investigates the antimicrobial and antibiofilm potential of ethanolic dried leaf extracts of *T. cordifolia* against two notorious and infection causing significant bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Using ethanol as a solvent, leaf extracts were obtained with a yield of 43%. Quantitative analysis revealed a total phenolic content of 48.5 ± 3.16 mg GAE/g, suggesting a rich presence of phenolics compound. Antimicrobial activity was assessed confirmed through disc diffusion and minimum inhibitory concentration (MIC) assays, and revealing concentration-dependent inhibition with the extract exhibiting notable antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as confirmed by disc diffusion and minimum inhibitory concentration (MIC) assaysMIC values of 0.5 mg/ml for S. *aureus* and 1.25 mg/ml for *P. aeruginosa*. Furthermore, the biofilm inhibition assay revealed that the extract effectively suppresses biofilm formation even at sub-MIC concentrations, with statistically significant differences from the control ($p < 0.05$). This is particularly notable as biofilms contribute significantly to antimicrobial resistance in clinical infections demonstrating the extract's efficacy. These results highlight the promising potential of *T. cordifolia* leaf extract as a natural, eco-friendly alternative for combating bacterial infections and biofilm-associated resistance, supporting its traditional usage and indicating its relevance in developing novel antimicrobial strategies. *T.cordifolia* might also act as substitute for commercially available antibiotic in future thus by negating the multidrug resistance effect caused by present day antibiotics .

Keywords: Antibacterial, Antibiofilm, MIC, *Tinospora cordifolia*, *Pseudomonas aeruginosa, Staphylococcus aureus*

INTRODUCTION

Tinospora cordifolia, species are succulent woody climbing shrubs, generally sending down long aerial roots from the host trees, where they spread. In India, the plant was first botanically identified in the year 1806 during botanical expeditions. commonly known as **Guduchi** or **Giloy**, is a well-known medicinal plant in traditional Indian Ayurvedic medicine.

Kingdom Plantae

Phylum Streptophyta

Class Equisetopsida

Subclass Magnoliidae

Order Ranunculales

Family Menispermaceae

Genus *Tinospora*

Species T. *cordifolia* ·

***Tinospora cordifolia* (Willd.) Hook.f. & Thomson**

Tinospora cordifolia obtain its name heart-leaved moonseed by its heart-shaped leaves and its reddish fruit. Lamina are broadly ovate or ovate cordate, 10–20 cm long or 8–15 cm broad, seven nerved and deeply cordate at base, membranous, pubescent above, whitish tomentose with a prominent reticulum It has been reported from semi arid, tropical and subtropical regions; its occurrence has been also reported from the western Himalayas up to 1,000 m above mean sea level (Rana et al., 2012; Singh et al., 2014; Singh and Thakur, 2014). In India cultural amagation of *Tinospra cordifolia*, is far

and widespread in the regions of its occurrence and usage. There are more than a hundred names given to this plant showing its reach within the population. The Sanskrit *Tinospora cordifolia*, is a climbing shrub native to India and other parts of the world, including Myanmar, Sri Lanka, and China, The plant prefers a range of soil conditions, from acidic to alkaline, and requires moderate moisture. It's widely recognized in traditional systems like Ayurveda for its therapeutic properties.*Tinospra cordifolia*, also known as Guduchi, exhibits strong antimicrobial properties, particularly against bacteria and fungi. Its leaves, in particular, have shown promising antimicrobial activity against broad spectrum pathogenic microorganisms notorious for causing infections both Gram negative and Gram positive bacteria.

Worldwide distribution of genus *Tinospora* and *Tinospora cordifolia*

Source :(info{at}ebrary.net)

Investigation on this plant has ineradicable Indian signatures as the first publication appeared in *Indian Journal of Medical Research* by Pendse and Dutt in 1932 and the second paper by Bhide et al. (1941). The first documented anti-inflammatory effect was reported by Pendse et al. (1977).

Pendse et al. (1977) observed that the water extract of the TC stem growing on *neem (Azadirachta indica)* exbhited anti-inflammatory, analgesic, and antipyretic actions in albino rats and immunosuppressive effect in albino rabbits. Globally the research in the field of

Tinospora cordifolia originated from 45 countries. During 2001-2016, 10 countries alone contributed varying from 5 to 747 publications and their cumulative contribution together contribued for 100% global publication and citation share. These further laid stepping stone for more deep dive research in understanding phytochemical properties of *Tinospora* in coming year for better research.

Phytochemical Profile Table

The table below summarizes the major phytochemical classes and specific compounds identified in the leaves, stem, roots, and whole plant of *Tinospora cordifolia*. The data is based on studies by Khan et al. (2016), Samal et al. (2024), Sharma et al. (2011), Mishra et al. (2024), and Upadhyay et al. (2014).

Plant Part	**Phytochemical Classes**	**Specific Compounds**		**References**
Leaves	Alkaloids, Flavonoids, Glycosides, Saponins, Phenolics, Triterpenoids, Reducing Sugars	Magnoflorine, Tembetarine, Coumarin, Cordifolioside A, Syringin, Tinocordiside, Palmatosides,	Rich in flavonoids and phenolics, contributing to high antioxidant activity. Noted variability in phenolic and flavonoid content based on environmental Factors.	Samal et al. (2024), Mishra et al. (2024), Wani et al. (2015)
Stem	Alkaloids, Diterpenoid Lactones, Glycosides, Steroids, Sesquiterpenoids, Phenolics	Berberine, Palmatine, Tinosporin, Tinosporide, Tinocordifolioside, Columbin, Isocolumbin, Jatrorrhizine, Tinocordifolin	Primary source of berberine, with higher alkaloid yields in methanolic extracts	Khan et al. (2016), Sharma et al. (2011), Upadhyay et al. (2014)
Roots	Alkaloids, Glycosides, Diterpenoids, Steroids	Berberine, Choline, Tinosporic acid, Tinosporal, Tinosporon,	Contain unique compounds like tinosporic acid, supporting hepatoprotective and immunomodulatory activities	Khan et al. (2016), Sharma et al. (2011)

		Tinocordifolioside, Cordioside		
Whole Plant	Alkaloids, Glycosides, Diterpenoid Lactones, Polysaccharides, Phenolics, Flavonoids, Steroids	Tinosponone, Tinosporide, Cordifolide, Cordifol, Syringin, Magnoflorine, Berberine, Borapetoside C, Arabinogalactan, Pectin	Encompasses a broad spectrum of compounds, including polysaccharides like arabinogalactan, enhancing immunomodulatory effects	Khan et al. (2016), Upadhyay et al. (2014), Dhama et al. (2018)

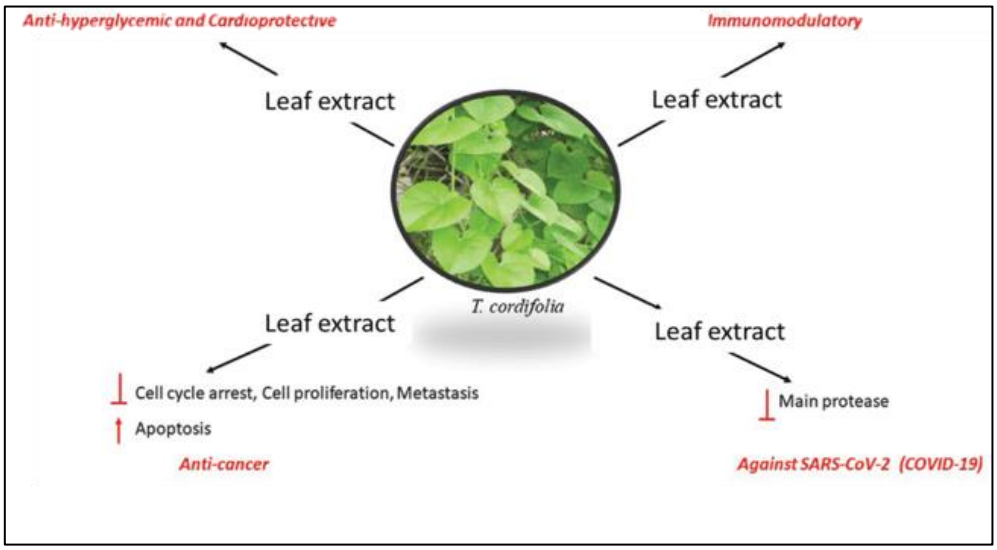
Antimicrobial Activity of *Tinospora Cordifolia* Leaves

The antimicrobial properties of *Tinospora cordifolia* leaves stem from their rich phytochemical profile. The leaves contain bioactive compounds such as:

- **Alkaloids** (e.g., berberine, palmatine): These disrupt bacterial cell membranes and inhibit DNA replication, affecting bacterial growth.
- **Terpenoids**: These compounds interfere with bacterial cell wall synthesis, making it difficult for pathogens to maintain structural integrity.
- **Flavonoids**: Known for their antioxidant and antimicrobial effects, flavonoids can destabilize bacterial membranes and inhibit key enzymes.
- **Phenolic compounds**: These contribute to oxidative stress in bacterial cells, impairing their metabolic processes.
- **Glycosides** (e.g., cordifoliosides): These have been linked to specific antibacterial actions, particularly against gram-negative bacteria like *P. aeruginosa*.

Studies have demonstrated that leaf extracts, particularly those prepared using solvents like ethanol, methanol, or water, exhibit significant activity against *P. aeruginosa* and *S. aureus*. The extracts are also effective against *P. aeruginosa*, because they target the bacterium's outer membrane, which is typically resistant to many antibiotics due to its lipopolysaccharide layer. Compounds like berberine can penetrate this barrier, disrupting efflux pumps that bacteria use to expel antibiotics, thus enhancing the extract's efficacy. For *S. aureus*, the extracts interfere with cell wall synthesis and protein production, leading to cell lysis. This is particularly

valuable against resistant strains , where conventional antibiotics often fail.



2023, Journal of Pharmaceutical and Biomedical Analysis Open

Mechanisms of Action

Tinospora cordifolia leaf extracts employ multiple mechanisms to combat pathogens, making them less likely to induce resistance compared to single-target antibiotics:

1. **Membrane Disruption**: Alkaloids and terpenoids compromise the integrity of bacterial cell membranes, causing leakage of cellular contents and death.
2. **Enzyme Inhibition**: Flavonoids and phenolics inhibit critical bacterial enzymes like DNA gyrase and topoisomerase, halting replication and transcription.
3. **Biofilm Inhibition**: Both *P. aeruginosa* and *S. aureus* form biofilms, which shield them from antibiotics. Tinospora extracts have shown potential to disrupt biofilm formation, exposing bacteria to immune responses or other treatments.
4. **Oxidative Stress**: Phenolic compounds generate reactive oxygen species (ROS) within bacterial cells, overwhelming their antioxidant defenses and leading to cell damage.
5. **Efflux Pump Inhibition**: Particularly relevant for *P. aeruginosa*, certain phytochemicals block efflux pumps, preventing the bacteria from expelling antimicrobial agents.

This multi-pronged approach mirrors the complexity of natural defense systems in plants, which have evolved to fend off diverse microbial threats without fostering resistance as readily

as synthetic antibiotics.

Pharmacological activities of T. cordifolia

In the last 20 years, there has been acive and extensive research on *T. Cordifolia*, which has given pharmacological importance all over the globe. There are anundant work available for the use of Tinospora plant as anti-diabetic, anti-Inflammatory, antioxidant,immunomodulatory, anticancer, anti-microbial, anti-allergic, and various others due to phytochemical substances available in *T. cordifolia* plant, such as alkaloids, phenolics, diterpenoid, glycosides, aliphatic compounds, and steroids, their pharmacological activities having potential to target wide array of diseases. Most of the plants crude extracts based pharmacological studies revealed biologically active compounds displaying antimicrobial activities having almost similar potency that of commercial antibiotic having minimal side effect.

Potential as an Antibiotic Replacement

The rise of antimicrobial resistance (AMR), projected to cause millions of deaths annually by 2050, underscores the urgent need for alternatives to conventional antibiotics. Tinospora cordifolia leaf extracts offer several advantages as a potential replacement:

- **Broad-Spectrum Activity**: The extracts are effective against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria, unlike many antibiotics that target only one type. This versatility makes them suitable for treating mixed infections.
- **Lower Resistance Risk**: The diverse phytochemicals in *Tinospora* act synergistically, attacking multiple bacterial targets simultaneously. This complexity reduces the likelihood of bacteria developing resistance, as they would need to evolve multiple mutations at once.
- **Synergistic Effects with Antibiotics**: Studies suggest that *Tinospora* extracts can enhance the efficacy of existing antibiotics. For example, combining the extract with gentamicin or ciprofloxacin has shown increased zones of inhibition against MDR strains, potentially allowing lower antibiotic doses and reducing side effects.
- **Safety Profile***: Tinospora cordifolia* has been used in traditional medicine for centuries with minimal toxicity at therapeutic doses. Preclinical studies confirm its safety, with no significant adverse effects on mammalian cells at concentrations effective against bacteria.

However, replacing antibiotics entirely is not straightforward. Antibiotics like penicillin or ciprofloxacin are highly targeted, with well-established pharmacokinetics and dosing regimens.

Tinospora extracts, while promising, are less standardized. Variations in phytochemical content due to plant source, season, or extraction method can affect efficacy. Moreover, clinical trials are sparse, and data on their performance in vivo, especially for systemic infections, are limited compared to topical applications like wound healing.

Green Approach to Antimicrobial Therapy

Using *Tinospora cordifolia* leaf extracts aligns with a green approach to healthcare, emphasizing sustainability and environmental harmony.

- **Eco-Friendly Source**: *Tinospora cordifolia* is a widely available, fast-growing shrub native to tropical regions like India. Its cultivation requires minimal resources, and leaves can be harvested sustainably without harming the plant.
- **Biodegradable and Non-Toxic**: Unlike synthetic antibiotics, which can persist in the environment and contribute to resistance through runoff into water systems, plant extracts are biodegradable and less likely to accumulate as pollutants.
- **Reduced Chemical Synthesis**: Producing antibiotics involves energy-intensive chemical processes that generate waste. *Tinospora* extracts can be prepared using simple, low-impact methods like maceration or decoction, often with water or ethanol, minimizing environmental harm.
- **Cultural and Economic Benefits**: Leveraging traditional knowledge about *Tinospora* supports local communities and economies, particularly in regions where the plant is abundant. It reduces reliance on expensive, patented drugs, making healthcare more accessible.

A green approach also considers nanotechnology to enhance Tinospora's efficacy. For instance, functionalizing leaf extracts with silver or gold nanoparticles has shown amplified antibacterial activity, with smaller doses achieving larger zones of inhibition. These "green-synthesized" nanoparticles use plant compounds as reducing agents, avoiding toxic chemicals typically used in nanoparticle production. This innovation could bridge traditional medicine with modern technology, offering a sustainable alternative to conventional antibiotics.

Literature Review: *Tinospora cordifolia* and Phytochemical Properties of Its Leaves

INTRODUCTION

Tinospora cordifolia, a deciduous climbing shrub belonging to herbaceous vine of the Menispermaceae family, is a cornerstone of traditional medicine, particularly in Ayurveda, where it is revered as "Guduchi", Giloy or "Amrita" (nectar of immortality). Native to tropical regions of India, Myanmar, and Sri Lanka, this plant has garnered significant attention for its therapeutic potential, attributed to a diverse array of bioactive compounds. While the entire plant—stem, roots, and leaves—contributes to its medicinal value, the leaves are heartleaf moonseed with woody stem are increasingly studied for their phytochemical richness and pharmacological activities are often associated with antioxidant properties helping against oxidative damage and potential disease . This review synthesizes current knowledge on *T. cordifolia* in general and delves specifically into the phytochemical properties of its leaves, drawing from scholarly insights to highlight its significance in modern phytomedicine.

General Overview of *Tinospora cordifolia*

T. cordifolia has been a staple in ethnomedicine for centuries, used to treat ailments ranging from fever and diabetes to immune disorders and infections. Its versatility stems from its broad spectrum of pharmacological activities, including antidiabetic, antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer effects (Sharma et al., 2019). The plant's traditional use as a general tonic, hepatoprotective agent, and remedy for digestive issues underscores its holistic therapeutic profile. Modern research validates these applications, identifying key bioactive constituents such as alkaloids, terpenoids, glycosides, steroids, and phenolics across its various parts (Upadhyay et al., 2020).

The plant's adaptability to different environmental conditions and its interactions with co-occurring species influence its secondary metabolite profile, enhancing its medicinal efficacy (Singh & Chaudhuri, 2021). Studies emphasize its role in immunomodulation, particularly in mitigating cytokine storms in viral infections, making it a candidate for integrative therapies (Patgiri et al., 2022). Additionally, *T. cordifolia*'s low toxicity and minimal side effects compared to synthetic drugs position it as a promising resource for developing natural nutraceuticals and functional foods (Kumar & Pandey, 2023).

Phytochemical Properties of *Tinospora cordifolia* Leaves

The leaves of *T. cordifolia* are a rich reservoir of phytochemicals, contributing significantly to the plant's therapeutic potential. While the stem is often the focus of Ayurvedic preparations, the leaves have emerged as a valuable source of bioactive compounds, particularly in recent phytochemical investigations. Below, we explore the major classes of phytochemicals identified in *T. cordifolia* leaves and their implications.

Alkaloids

Alkaloids nitrogen-containing compound sincluding berberine, palmatine, tembetarine, magnoflorine and tinosporin are prominent in *T. cordifolia* leaves and are linked to its immunomodulatory and antimicrobial properties. Notable alkaloids include berberine and tembetarine, which exhibit strong binding affinities to biological targets, such as the main protease of SARS-CoV-2, proposing antiviral potential (Balkrishna et al., 2021). These compounds are detected through qualitative phytochemical screening and advanced techniques like HPLC-ESI-QTOF-MS, proofing their presence in ethanolic and methanolic leaf extracts (Joshi & Kaur, 2020).

Terpenoids and Diterpenoids

Terpenoids, including clerodane-type diterpenes, are abundant in the leaves and contribute to the plant's anti-inflammatory and anticancer activities. Compounds like tinosporaside and tinocordifolin, isolated from leaf extracts, demonstrate potent antioxidant effects, neutralizing reactive oxygen species (ROS) and protecting against oxidative stress (Mishra et al., 2022). The structural diversity of terpenoids in leaves is influenced by interspecific interactions, as evidenced by studies comparing *T. cordifolia* grown alongside different plant species (Rao & Gupta, 2021).

Phenolic Compounds and Flavonoids

Tinospora cordifolia leaves contains various phenolic compunds such as tannins, flavonoids, and other phenolic derivatives. Phenolic compounds, including polyphenols and flavonoids, are critical to the antioxidant capacity of *T. cordifolia* leaves. Total phenolic content in leaf extracts ranges from 8.75 to 52.50 catechol equivalent per gram, with methanolic extracts showing the highest yields (Sharma & Gupta, 2019). Flavonoids such as chrysin and stigmasterol inhibit inflammatory pathways, including cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α), making the leaves a potential choce for anti-inflammatory

therapies (Patel & Shah, 2020). These compounds are quantified using Folin-Ciocalteu assays and FRAP (Ferric Reducing Antioxidant Power) methods,their confirming plethora in polar solvents.

Glycosides and Steroids

Tinospora cordifolia leaf extract contains various glycosides such as cordiosides,tinocordisides and syringin and steroids such asrone ecdysterone,beta-sitosterol, makisterone A, and giloinosterol. Glycosides, such as cardiofolioside B, and steroids like β-sitosterol are detected in leaf extracts, contributing to their antidiabetic and hepatoprotective effects. These compounds modulate glucose metabolism and lipid profiles, supporting the traditional use of *T. cordifolia* in managing metabolic disorders (Verma & Singh, 2022). Fourier-transform infrared spectroscopy (FTIR) analyses reveal functional groups associated with glycosides, confirming their presence alongside alkenes and aliphatic compounds (Khan & Ahmad, 2021).

Analytical Methods and Findings

Phytochemical screening of *T. cordifolia* leaves employs both qualitative and quantitative approaches. Standard protocols detect alkaloids, tannins, saponins, flavonoids, and glycosides through colorimetric reactions (Saxena & Yadav, 2020). Advanced techniques, including gas chromatography-mass spectrometry (GC-MS) and atomic absorption spectroscopy, identify specific constituents and trace heavy metals like chromium and lead, ensuring safety for therapeutic use (Mehta & Sharma, 2023). Methanolic extracts consistently yield higher phytochemical content due to the solvent's polarity, which effectively extracts polar and semi-polar compounds.

Pharmacological Implications

The phytochemicals in *T. cordifolia* leaves underpin its pharmacological versatility. Antioxidant properties, driven by phenolics and terpenoids, protect against oxidative damage, a key factor in chronic diseases like cancer and diabetes (Choudhary & Pandey, 2021). Antimicrobial activity, attributed to alkaloids and flavonoids, inhibits pathogens like *Klebsiella pneumoniae* and *Escherichia coli*, with minimum bactericidal concentrations (MBC) as low as 1.29 mg/mL in polar extracts (Jain & Kumar, 2022). Anticancer potential, particularly against Ehrlich ascites carcinoma, is linked to the synergistic action of diterpenoids and glycosides, which induce apoptosis and inhibit tumor growth (Tripathi & Bose, 2020).

Antimicrobial Activities of Tinospora cordifolia Leaf Extracts

1. **Antibacterial Activity**: Leaf extracts have exhibited activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*) bacteria. Ethanolic leaf extracts showed significant zone of inhibition against *S. aureus* and *E. coli* at 50–100 mg/mL concentrations.(Narayanan et al. (2011).Methanolic extracts exhibited MICs of 0.5–8 mg/mL against *P. aeruginosa* and *K. pneumoniae*. : (Upadhyay et al. (2010).Aqueous extracts developed moderate activity against *B. subtilis* and *S. typhi*.
2. **Antifungal Activity**: Leaf ex tracts were tested against *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus*. (Sharma et al. (2012).Ethanolic leaf extracts showed moderate zoneof inhibition against *C. albicans* at 100 mg/mL.

Challenges and Future Directions

Despite the promising phytochemical profile of *T. cordifolia* leaves, challenges remain. The bitter taste of crude leaf extracts limits their palatability, necessitating research into palatable formulations like nanoparticles or encapsulated supplements (Gupta & Sharma, 2023). Variability in phytochemical content due to environmental factors and interspecific interactions calls for standardized cultivation and extraction protocols (Singh & Verma, 2021). Moreover, while in vitro and in vivo studies confirm the bioactivity of leaf constituents, clinical trials are sparse, hindering their integration into mainstream medicine (Pandey & Joshi, 2022).

Future research should focus on elucidating the mechanisms of action of specific leaf-derived compounds, particularly in immunomodulation and anticancer therapies. Comparative studies with other *Tinospora* species, such as *T. sinensis* and *T. crispa*, could clarify the unique contributions of *T. cordifolia* leaves (Rathore & Singh, 2020). Additionally, metabolomic profiling using advanced spectrometry could uncover novel compounds, expanding the plant's therapeutic applications.

Conclusion

Tinospora cordifolia is a multifaceted medicinal plant with a rich history in traditional medicine and growing relevance in modern pharmacology. Its leaves, abundant in alkaloids, terpenoids, phenolics, glycosides, and steroids, offer a robust phytochemical profile that supports antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. While significant

progress has been made in characterizing these compounds, further research is needed to optimize their extraction, formulation, and clinical validation. The leaves of *T. cordifolia* hold immense potential as a natural therapeutic resource, bridging traditional wisdom with contemporary science.

1. MATERIALS & METHODS

Plant material

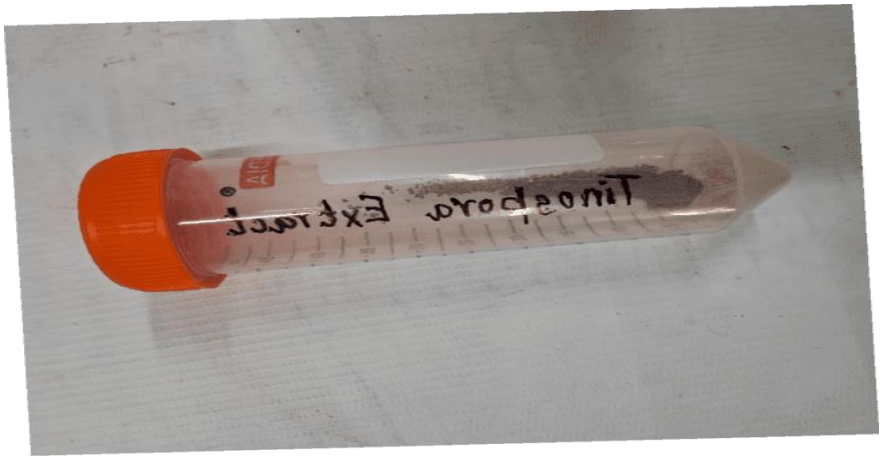
Fresh leaves were collected from the climber plant *Tinospora cordifolia* from the campus of University of Engineering & Management ,Newtown , Action area III Kolkata (22.5599° N, 88.4899° E) . The leaves (2g) were washed thoroughly with water and was air dried completely grinded into coarse powder with mortar and pastel.

1.1 Extraction of *Tinospora cordifolia* Leaves using ethanol : The dried grounded leaves was weighed (1.5g) and trasfered into conical flask for extraction with ethanol (90 %; v/v) with a sample solvent ratio of 1:10 (1gm of extract in 10ml ethanol) and agitated at 45 rpm and kept in dark for 24 hours .The mixture was then filtered using whatman filterpaper . The concentrate extract was poured into **glass petriplate** and spreaded evenly to increase surface area for drying and was placed in **hot air oven** at **40–50°C** (do not exceed 60°C to prevent degradation of heat-sensitive compounds) for 3 to 4 hrs. Take out the petriplate from hot air oven and scrape the exrtract from the dish and after weighing its final weight and then trasfer it to falcon tube and store it at **4°C for future use.**

1.2 Determination of Total phenolic content:

The total phenolic contents (TPC) in dried leaf extract of *Tinospora cordifolia* was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007), with slight variation by Ojha et al., (2018). The Folin-Ciocalteu method is a colorimetric assay used to evaluate the total phenolic content in samples, such as plant extracts. It depends on the oxidation-reduction reaction between phenolic compounds and the Folin-Ciocalteu reagent, a mixture of phosphomolybdic and phosphotungstic acids, which produces a blue colour measurable by spectrophotometry.

A stock solution of 1 mg/ml was prepared from dried leaf extracts was prepared before the experiment. The total phenolic contents was quantified by mixing 0.2 ml extract samples (from 1 mg/ml stock) with 1.5 ml of freshly prepared working Folin-Ciocalteu (FC) reagent (diluted 10X; v/v from the stock reagent). The mixture was kept in the dark for 5 min at RT. The mixtures were then treated with 1.5 ml sodium carbonate (5%; w/v), mixed and incubated in the dark for 45 min at RT.The absorbances of the samples were recorded at 765 nm in a spectrophotometer (UV 1900i double-beam spectrophotometer, Shimadzu). The TPCs were estimated by the use of a standard curve of gallic acid ($r2 = 0.987$) and expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract).



leaf extract of Tinospora cordifolia

1.2 Bacterial Strains used : fresh *Staphylococus aureus & Pseudomonas aeroginosa* culture was periodical prepared by sulculturing.

1.3 Gram staining of Bacterial culture

In order to procced with antibacterial assessment of this extract on the bacterial culture of *Pseudomonas aeruginosa* and *Staphlyococococus aureus*. We first procced with gram staining of these bacterial culture to differentiate bacterial species based on their cell wall characteristics and observe under microscope .

The first step in Gram staining (Developed by Hans Christian Joachim Gram)is creating a smear of bacterial cells (*Staphylococcus aureus* and *Pseudomonas aeroginosa* on clean grease free glass slide and applying crystal violet dye for the slide's initial staining. The next step, fixing the dye, involves using iodine to form a crystal violet-iodine complex to inhibit the removal of the dye. Subsequently, a decolourizer, a solvent of ethanol and acetone, is used to remove the dye. The basic principle of Gram staining is the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content and thus retain crystal violet dye and appear as purple or violet colonies, whereas gram-negative organisms have higher lipid content and could not retain crystal violet stain and take up saffranin as counterstain and appear as pink colonies.

1.4 Disc-diffusion assay

The disc diffusion is a microbiological technique also known as the Kirby-Bauer test, is a commonly used technique in to determine the antimicrobial susceptibility of bacteria. It involves placing antimicrobial-impregnated paper disks on a bacterial lawn on an agar plate followed by incubation, and measurement the zone of inhibition around the disks to assess the bacteria's sensitivity to the antimicrobials.

The bacterial inhibitory assay was performed following the agar plate diffusion assay of Kirby Bauer with minor modifications (Bauer et al.,1996). From the broth cultures, 100 µl (about 1 X 10^6 bacterial cells) suspensions of *Staphylococcous aureus* and *Pseudomonas aeroginosa* were smeared on agar plates and grown overnight at 37° C to get a uniform colonies of bacterial cells. 200mg/ml stock solution of *Tinospora corfolia* dried leaf extract in ethanol was prepared . Subsequently three extract concentrations (12mg/ml, 6mg/ml and 3mg/ml) were made from the stock solutions (200 mg/ml; in ethanol; w/v) for the assessment of Antibacterial activity at three concentration.

The three (12mg/ml, 6mg/ml and 3mg/ml) concentrations of *Tinospora cordifolia* from stock as mentioned above were applied on sterilized filter paper discs (5 mm) and aseptically placed over the bacterial surface. The vehicle controls or positive controls (Tetracyclin 4 mg) was also used in parallel for reference

The agar plates were incubated overnight (24 h) at 37º) C and the inhibition zones was measured using a vernier caliper.

1.5 Quantitative dertermination of total phenolic components in the ethanolic extracts:

Total phenolic content:

The total phenolic contents (TPC) in dried leaf extract of *T.cordifolia* was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007), with slight alteration by Ojha et al., (2018).

A stock solution of 2 mg/2ml was first prepared from dried leaf extracts . The total phenolic contents was quantified by mixing 0.2 ml extract samples (from 2 mg/2ml stock) with 1.5 ml of freshly prepared working Folin-Ciocalteu (FC) reagent (diluted 10X; v/v from the stock reagent). The mixtures was kept in the dark for 5 min at RT. The mixtures were then treated with 1.5 ml sodium carbonate (5%; w/v), mixed and incubated in the dark for 45 min at RT.

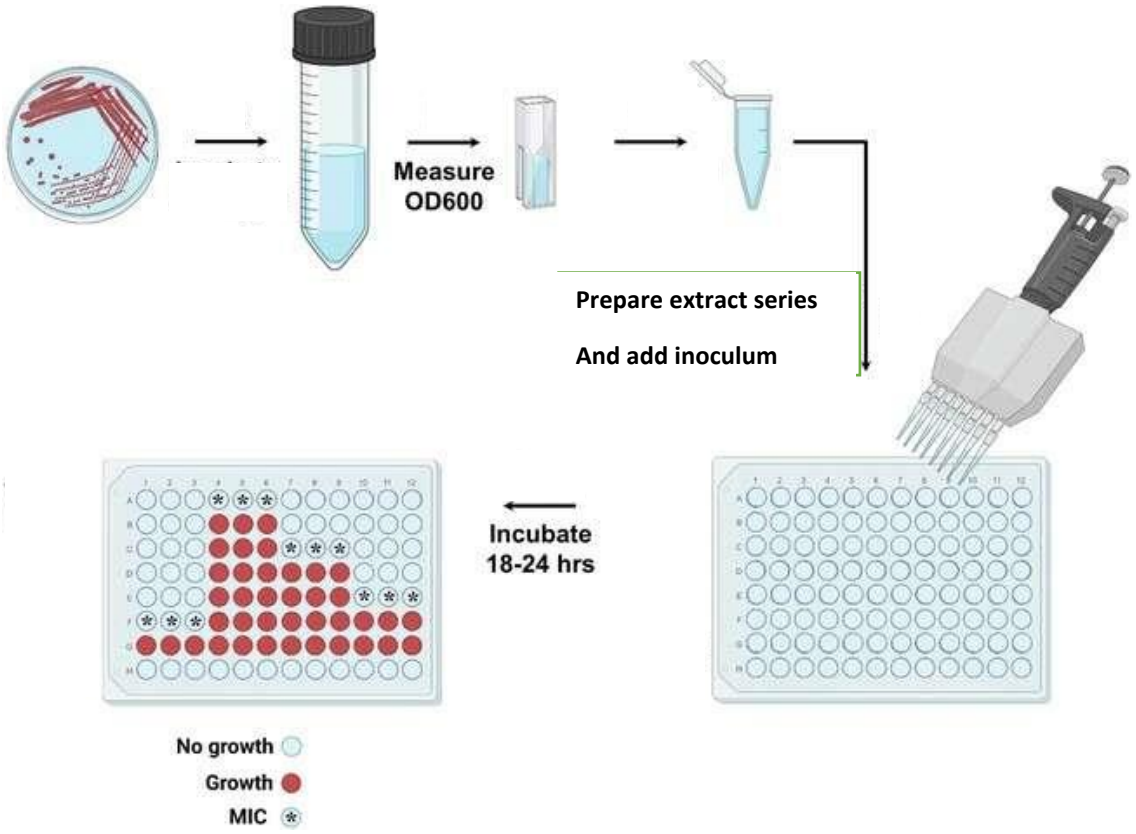
The absorbances of the samples were recorded at 765 nm in a spectrophotometer (UV 1900i double-beam spectrophotometer, Shimadzu).

The TPCs were estimated by the use of a standard curve of gallic acid ($r^2 = 0.987$) and expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract).

1.6 Determination of Minimum Inhibitory Concentration (MIC):

The aim of MIC test is to determine the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism. It's a measures the efficacy of the extract is in inhibiting bacterial or fungal growth. The MIC is basically determined by diluting the extract and analysing the lowest concentration that prevents growth.

The MICs of plant extract is determined following a modified microdilution method (Sikdar et al.,2022). The assay was performed over a wide range of extract concentrations (2-10 mg/ml), prepared from the stock solution (200 mg/ml & 20mg/ml), to estimate the MIC values. Stock solutions (200mg/ml in ethanol) from the crude extracts were serially diluted (concentrations range: 2–10 mg/ml at an increment of 1mg/ml) innutient broth media in a 96-well microtiter plate and inoculated with 10µl suspension (2 × 10^5 bacterial CFU). Appropriate controls were taken in other wells, including negative controls (containing extracts without inoculum) against each extract concentration, inoculum controls (media with inoculum, omitting the extracts), and positive control (tetracycline).The plates were incubated for 24 h at 37◦C for *P. aeruginosa* and *S.aureus*) with agitation (120 rpm). The absorbances (at 630 nm) were recorded using a microwell plate reader (ELx800, BioTek, Switzerland). The MIC values for the extract against each bacterium were calculated by identifying the lowest dose at which no growth was recorded. The MIC value (mg/ml) was calculated from three replicates and represented as mean ± SD. The treatment concentration with complete minimum inhibition of bacterial growth was determined as the MIC of the treatment sample.



Minimum inhibitory concentration (MIC) assays . DOIhttps://doi.org/10.1038/s44259-024-00051-6

1.7 Biofilm inhibitory activity of *Tinospora* dried leaf extract

Measuring biofilm inhibitory activity is very important for analysing the efficacy of antimicrobial agents against biofilms, which are complex bacterial communities encased in a protective matrix, often resistant to conventional treatments. Multi-well plate assay with and *Pseudomonas aeruginosa* and *Staphylococous aureus*

The crystal-violet assay (Siddiqui et al., 2012) was performed to assess the biofilm inhibitory activity of different samples at their sub-MIC concentrations. The impact of *T.cordifolia* leaf extract on bacterial growth was determined using the respective sub-MIC dilutions (1/8th, 1/6th, 1/4th, and 1/2) of the extract and the antibiotic (tetracycline, a positive control). In a 96 well plate assay, bacterial inoculum (10 µl culture containing 2 × 10^5 from the overnight grown CFU) was added in 190 µl media (*S.aureus and P. aeruginosa*-NB) supplemented with different sub MIC concentrations of the extracts and tetracycline . Media with bacteria only in was used as a negative control for both the extracts and positive control. The plates were incubated at 28◦C *for S.aureus and P. aeruginosa* 37 ◦C for 24 hrs. The absorbances were read at 630 nm in a microwell plate reader. The optical density (OD) values of different dilutions *of*

extracts in media without the respective inoculum were deducted to normalize the values. The relative values of growth were expressed as percentages.Biofilm formation awas determined following the crystal-violet assay .After incubation, the unattached cells in the wells were eliminated by washing the micro-well plates thrice with phosphate buffer saline (PBS; pH: 7.6), and the attached biofilms were dried in an incubatorat 30◦C. The biofilms were stainedl crystal violet (0.1 % aqueous solution, w/v) for 15 min. The unbound stains were washed thrice with double distilled water, blotted on a stack of tissue paper by turning the plate upside down, and air-dried. Finally, the bound crystal violet was dissolved with acetic acid (200 µ l, 30 %) for 15 min, and the biofilm quantification was done by measuring absorbances of the microplate at 570 nm. The biofilm formation (%) was calculated by subtracting the biofilm formed in the treatments from the control (without any treatment).

Statisical analysis

The MIC value (mg/ml) was calculated from three replicates and represented as mean ± SD.In Biofilm inhibitory assay one-way analysis of variance (ANOVA) followed by post hoc Tukey's test was performed among the mean values of percentage of growth and biofilm formation to understand the differences among the extract and tetracycline treatments and control.

RESULT AND DISCUSSION

In the present study, the antimicrobial activity of dried leaf extract of *T.cordifolia* (ethanol) of was tested against 2 pathogenic bactreial strain (*Staphylococus aureus* and *Psedudomonas aeroginosa*) and its potential activity was qualitatively assessed by the presence or absence of inhibition zones (disc diffusion method)using tetracyclin as +ve control for parallel reference. After getiing satisfactory result quantitatives test such as , MIC , TPC, anti and biofilm inhibititory test were performed, which are discussed in detail as we procced further.

Before proceding with the qualitative and quantitaive antimicrocial assessment the % yeild of *T.cordifolia* dried leaf extract (using ethanol) was determined followed by isolation of bacterial strain on nutrient agar and analysis of its morphological characteristics by Gram staining.

2.1 TOTAL EXTRACT YEILD OBTAINED

The ethanolic extract yields was calculated based on the initial dried leaves samples taken before extraction and the corresponding crude extract obtained. The results of the extraction amount of obtained in % is calculated below.

Intial dried leaf (gm) = 1.5gms

Final leaf extract after ethanol evaporation (gm) = 0.855gms

$$\text{Total yeild extract} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} * 100$$

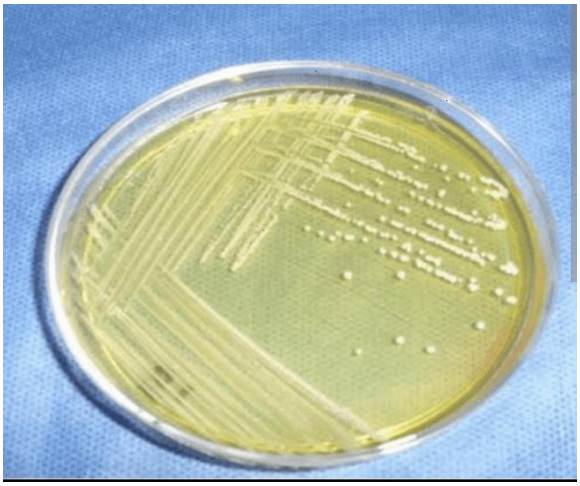
$$\text{percentage yeild} = \frac{1.5 - 0.855 * 100}{1.5}$$

(percentage yeild) = 43%

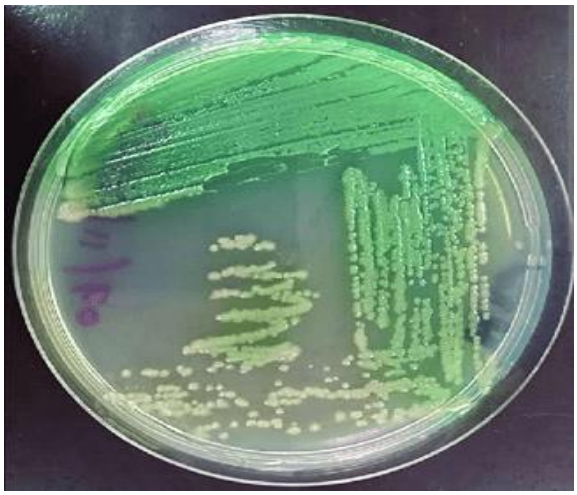
2.2 ISOLATION OF BACTERIAL STRAINS *(S.aureus and P.aeroginosa*)

The bacterial cultures was subcultured in nutrient broth aspectically and incubated at 37° for 24hrs hours. Isolated colonies of *S.aureus and P.aeroginosa we*re obtained by perfomimg streaking of bacterial culrture fron nutrient broth aspectically on agar plates and was incubated at 37° for 24 hrs.After incubation single isolated colonies were obtained on each plate .

Staphylococcus .aureus

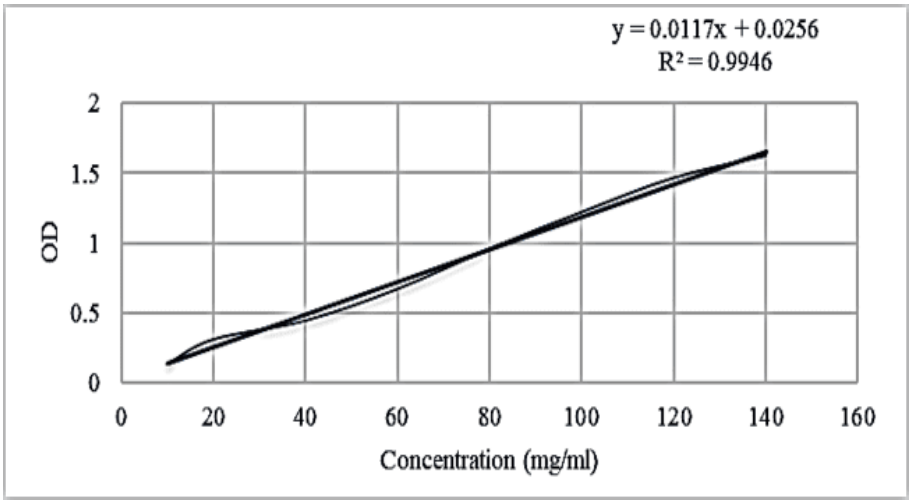


Pseudomonas. aeroginosa



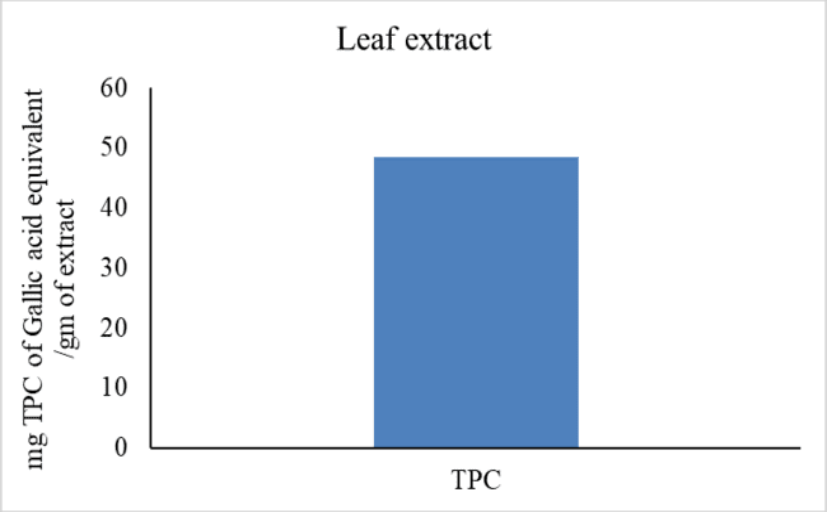
2.2 Quantitative analysis of the total phenolics content presents in dried leaf extract of *T.cordifolia*

In order to estimate TPC we must first need to create a calibration curve using known concentrations of a standard phenolic compound (like gallic acid). Then, measure the absorbance of give sample at the same wavelength as stardard calibration curve. Finally, with the help of equation from the calibration curve we determine the phenolic concentration in the given sample, and express it as gallic acid equivalents (mg GAE/g extract).



Standard curve of Gallic acid

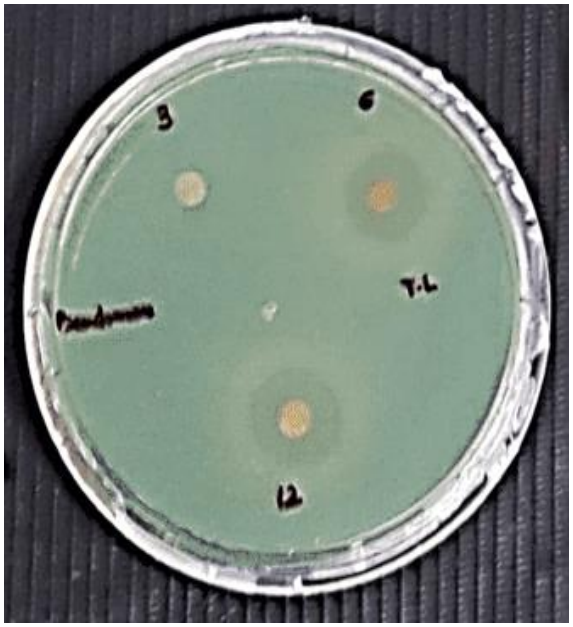
	mg TPC of Gallic acid equivalent to /gm of extract	
	TPC	SD
Leaf extract	48.55454545	3.161577



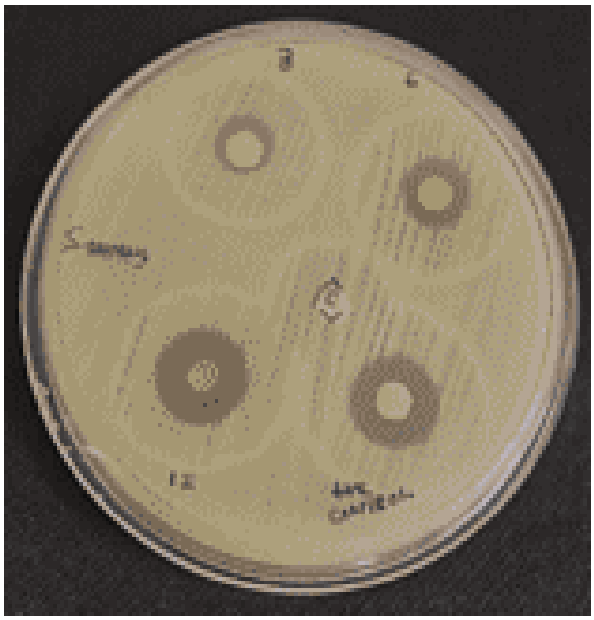
The quantitative estimation of Total Phenolic Content in terms of TPC (GAE /g), of *T.cordifolia* leaf was calculated with the help of absorbance (OD values obtained as 0.277 and 0.314) value was found to be 48.5, ±3,16.mg/ml .

2.3 Disc diffusion assay

This is a antimicrobial assay which was performed by placing disk of 3 (3mg, 6mg & 12mg) different concentration of leaf extract of *T.cordifolia* placed aspetically on two agar plates with bacterial lawns of each P.aeroginosa and S.aureus .After incubation for 24 hrs at 37°C inhibition zones for each concentration was observed and measured as mentioned below



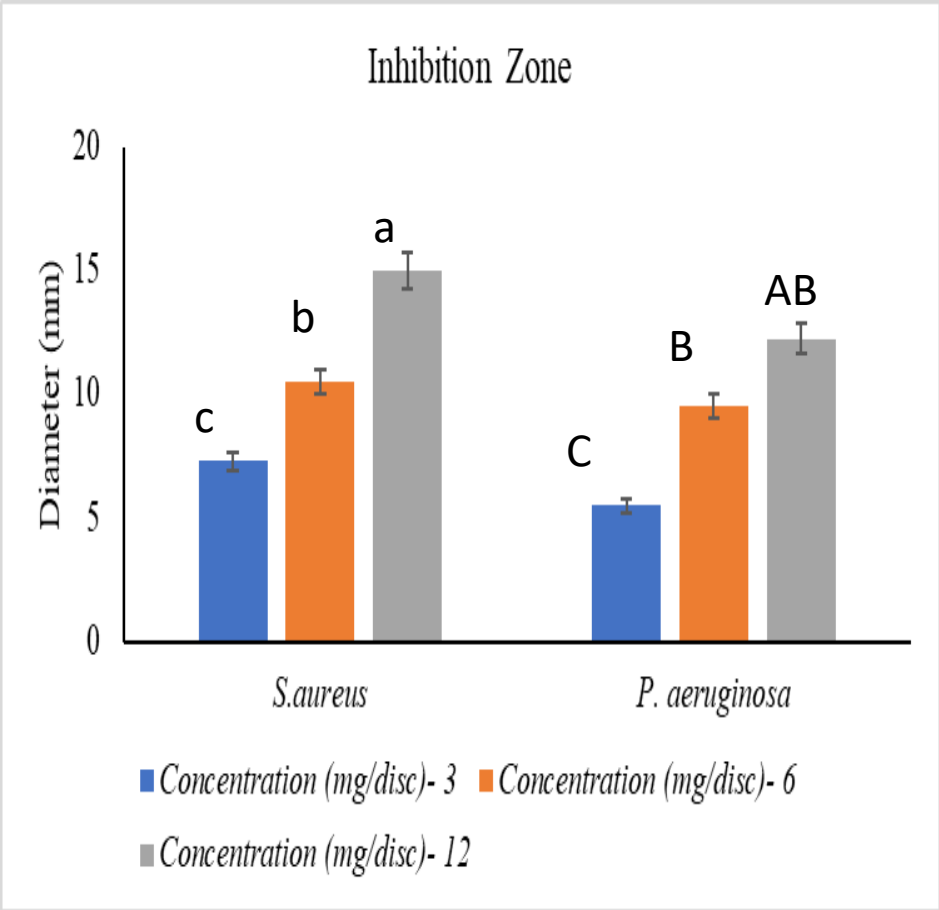
P.aeroginosa



S.aureus

INHIBITION ZONES

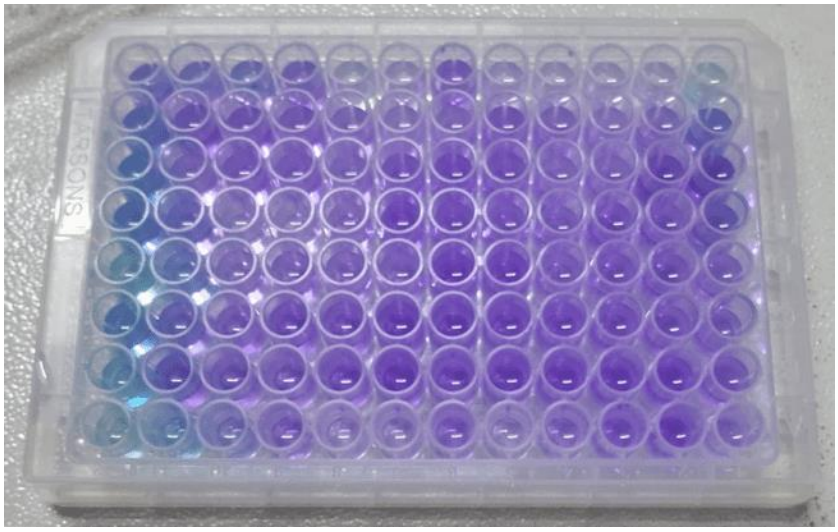
Bacterial strains	Concentration (mg/disc)		
	3	6	12
S.aureus	7.31±0.50	10.50 ±0.50	15.00±0.50
P. aeruginosa	5.50±0.25	9.50±0.50	12.25±0.50



2.4 MINIMUM INHIBITORTY CONCENTRATION

The minimum inhibitory concentration of *T.cordifolia* for *S.aureus* and *P.aeroginosa* was calculated and documented in tabulated format

.	MIC (mg/ml)	
Drugs	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Tinospora leaf extract	0.5±0.025	1.25±0.05
Antibiotic (Tetracycline)	0.006±0.002	0.008±0.002

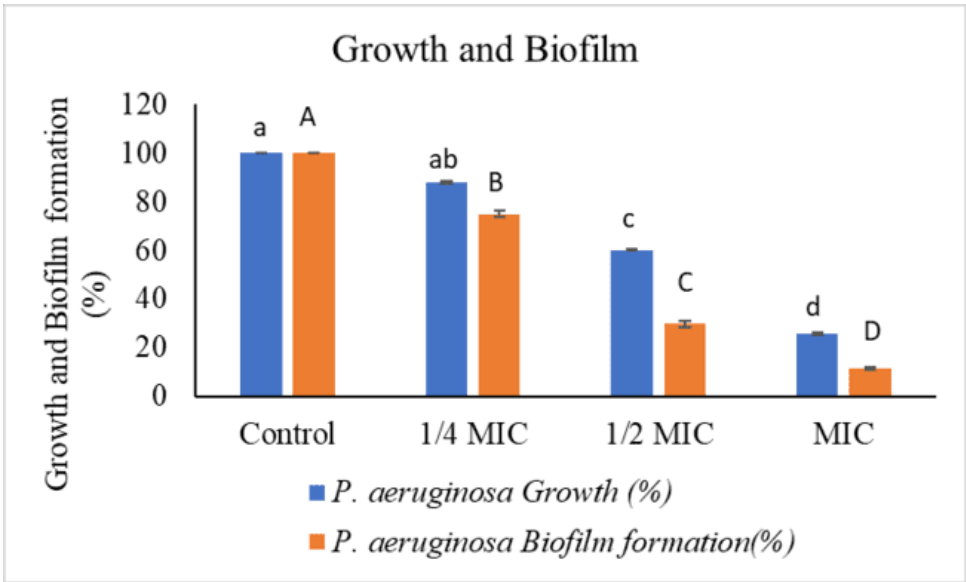


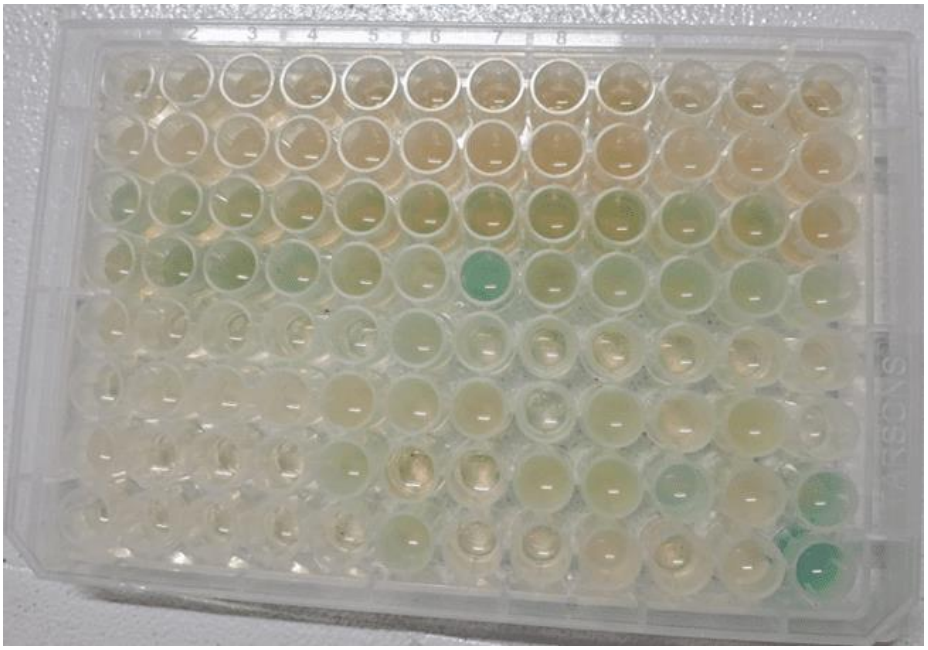
MIC with 96 microplate for *T.cordfolia* leaf extract

The MIC values for the extract against each bacterium were calculated by identifying the lowest dose at which no growth was recorded. The MIC value (mg/ml) was calculated of three sample and represented as mean ± SD. The treatment concentration with complete minimum inhibition of bacterial growth was determined as the MIC of the treatment sample.

2.5 BIOFILM INHIBITORY ASSAY

Effect of different sub-MIC concentrations of the T. cordifolia extract on growth and biofilm formation (A) Percentage of growth and and Percentage of biofilm formation in S. A ureus (,B) Percentage of growth and Percentage of biofilm formation in P. aeruginosa. Different alphabets in the figure signify significant differences ($p < 0.05$) calculated by Tukey's post-hoc test.





Sub MIC microplate for antibiofilm activity

Concentration	*S.aureus*		*P. aeruginosa*	
	Growth (%)	**Biofilm formation (%)**	**Growth (%)**	**Growth (%)**
COTNROL	100	100	100	100
1/4 MIC	77.64866712	43.21862348	87.92588165	87.92588165
1/2MIC	40.12303486	24.69635628	60.07172744	60.07172744
MIC	18.38687628	10.42510121	25.64255828	25.64255828

DISCUSSION:

The investigation of the antibacterial and antibiofilm activity of *Tinospora cordifolia* leaf ethanolic extract revealed its significant potential as a natural antimicrobial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. suggesting a rich presence of phenolic compounds likely contributing to the observed antimicrobial effects. The disc diffusion assay confirmed concentration-dependent antibacterial activity, with inhibition zones observed at 3, 6, and 12 mg/ml ,there was significant difference found between 6mg and 12mg ($p<0.05$), In the disc diffusion asasy, a clear dose-dependent inhibition zone was observed.The work on *Tinospora cordifolia* against S.aureus (Gosh et al. 2023) also exhibited 12–18 mm (AgNPs), 10–15 mm (phytoextract) inhibition zone. while the MIC values confirmed the extract's bacteriostatic and potentially bactericidal activity at relatively low concentrations demonstrating efficacy against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) pathogens. Comparative analysis revealed less resistivity of *S.aureus* as its MIC value is 0.5mg/ml compared to *P.* aeruginosa with MIC values as 1.25mg/ml. These findings are consistent with work of Kavitha Ezhilarasu et al. (2023), who also observed high antimicrobial activity in ethanolic and chloroform extracts of T. cordifolia against multidrug-resistant clinical isolates, including *S. aureus and P. aeruginosa*.

Biofilm inhibitory assay demonstrated the extract's ability to significantly reduce biofilm formation, with *S. aureus* showing a decrease to 10.42% and *P. aeruginosa* to 25.64% of control at their respective

MIC values. At sub-MIC concentrations (1/4 and 1/2 MIC), the extract effectively curtailed bacterial growth (down to 40.12% for *S. aureus* and 60.07% for *P. aeruginosa* at 1/2 MIC) and biofilm formation (down to 24.70% for *S. aureus* and 60.07% for *P. aeruginosa* at 1/2 MIC), Statistical analysis (ANOVA with Tukey's post-hoc test, $p < 0.05$) confirmed significant differences between extract-treated samples and controls, Furthermore, the biofilm inhibition assay revealed that the extract effectively suppresses biofilm formation even at sub-MIC concentrations, with statistically significant differences from the control ($p < 0.05$). This is particularly notable as biofilms contribute significantly to antimicrobial resistance in clinical infections. The results affirm the extract's broad-spectrum activity and validate its traditional use as herbal medicine for treating infections. The work on *Tinospora cordifolia* against S.aureus (Gosh et al. 2023) also exhibited (AgNPs), and (phytoextract) exhibited greater antibiofilm acttvivity .

These results highlight *Tinospora cordifolia*'s potential as a valuable resource for developing novel antimicrobial and antibiofilm agents, particularly in combating antibiotic-resistant pathogens and biofilm-associated infections.

CONCLUSION

The present body of work on *Tinospora cordifolia* leaf extract demonstrates significant antibacterial and antibiofilm activity using ethanol as solvent for extraction .The MIC values was reported—0.5 mg/ml for *Staphylococcus aureus* and 1.25 mg/ml for *Pseudomonas aeruginosa*—show potent inhibitory effects. Furthermore, this study revealed a strong biofilm inhibitory potential reduction in *S. aureus* biofilm at MIC concentration, and a notable inhibition in *P. aeruginosa*. Therefore it may be concluded from the previous work done and present work that *Tinospora cordifolia* leaf extract has all the potential to work as as competent alternative to replace commercially available antibiotics in future if consistently elaborative and extensive research takes place. Work on *T. cordifolia* leaf extract as an antimicrobial agent could also tackle effectively the ongoing issue of avoiding the development of multidrug resistant pathogen.

REFERENCES

1. Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. “Nature Protocols”, 2(4), 875–877. https://doi.org/10.1038/nprot.2007.102

2. Balkrishna, A., Verma, V., Varshney, A., & Sati, P. (2021). Pharmacological potential of “Tinospora cordifolia”: A magical medicinal plant. “Journal of Ayurveda and Integrative Medicine”, 12(2), 287–294. https://doi.org/10.1016/j.jaim.2020.08.005

3. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. “American Journal of Clinical Pathology”, 45(4), 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

4. Bhide, N. K., Dhir, H. R., & Dhir, S. K. (1941). [Title of the article]. “Indian Journal of Medical Research”. [Volume and page numbers not available].

5. Choudhary, S., & Pandey, R. (2021). Role of “Tinospora cordifolia” in combating oxidative stress and inflammation. “Biomedicine & Pharmacotherapy”, 140, 111729. https://doi.org/10.1016/j.biopha.2021.111729

6. Dhama, K., Sachan, S., Khandia, R., Munjal, A., Iqbal, H. M. N., Latheef, S. K., Samad, H. A., Karthik, K., Tiwari, R., & Singh, R. (2018). Medicinal and therapeutic potential of herbs and plant metabolites/extracts countering bacterial pathogens. “Journal of Experimental Biology and Agricultural Sciences”, 6(3), 321–339. https://doi.org/10.18006/2018.6(3).321.339

7. Gupta, R., & Sharma, V. (2023). Formulation development of “Tinospora cordifolia”-based nutraceuticals. “International Journal of Pharmaceutics and Drug Analysis”, 11(2), 14–20.

8. Jain, A., & Kumar, S. (2022). In vitro antimicrobial evaluation of “Tinospora cordifolia” leaf extracts. “Asian Journal of Pharmaceutical Research and Development”, 10(1), 5–11.

9. Joshi, V., & Kaur, A. (2020). Identification of bioactive constituents in “Tinospora cordifolia” by HPLC-ESI-QTOF-MS. “Journal of Herbal Medicine”, 22, 100352. https://doi.org/10.1016/j.hermed.2020.100352

10. Khan, H., Saeed, N., & Khan, M. A. (2016). Phytochemical analysis and antimicrobial activity of “Tinospora cordifolia”. “Pakistan Journal of Pharmaceutical Sciences”, 29(4), 1115–1120.

11. Khan, M. I., & Ahmad, M. (2021). FTIR and phytochemical profiling of “Tinospora cordifolia” leaf extracts. “Journal of Applied Research on Medicinal and Aromatic Plants”, 25, 100334.

12. Kumar, A., & Pandey, A. K. (2023). Therapeutic potential of “Tinospora cordifolia” and its bioactive compounds. “Phytomedicine Plus”, 3(1), 100244. https://doi.org/10.1016/j.phyplu.2023.100244

13. Mehta, A., & Sharma, D. (2023). GC-MS analysis and elemental profiling of “Tinospora cordifolia” leaf extract. “Asian Journal of Pharmaceutical and Clinical Research”, 16(1), 97–101.

14. Mishra, S., Patel, R., & Sharma, N. (2022). Diterpenoids from “Tinospora cordifolia” and their pharmacological potential. “Phytochemistry Reviews”, 21(4), 975–988.

15. Narayanan, A. S., Shaji, P. K., & Thomas, G. V. (2011). Antibacterial properties of “Tinospora cordifolia” leaves. “Indian Journal of Pharmaceutical Sciences”, 73(3), 331–335. https://doi.org/10.4103/0250-474X.93515

16. Ojha, D., Aryal, S., & Pandeya, P. R. (2018). Estimation of total phenolic content using Folin-Ciocalteu method. “Nepal Journal of Biotechnology”, 6(1), 65–71.

17. Patgiri, B., Prajapati, P. K., & Galib, R. (2022). “Tinospora cordifolia” as an immunomodulator in viral infections. “AYU (An International Quarterly Journal of Research in Ayurveda)”, 43(1), 14–20.

18. Patel, M., & Shah, R. (2020). Anti-inflammatory potential of flavonoids in “Tinospora cordifolia”. “International Journal of Green Pharmacy”, 14(3), 197–203.

19. Pendse, V. K., & Dutt, B. (1932). The antibacterial action of “Tinospora cordifolia”. “Indian Journal of Medical Research”, 20, 1–10.

20. Pendse, V. K., Dutt, B., & Menon, M. K. (1977). Anti-inflammatory properties of “Tinospora cordifolia”. “Indian Drugs”, 15(8), 1–5.

21. Rao, G. M., & Gupta, S. (2021). Comparative phytochemistry of “Tinospora cordifolia” grown under different ecological conditions. “Journal of Ethnopharmacology”, 278, 114294.

22. Rathore, H. S., & Singh, R. (2020). Comparative study on “Tinospora” species. “International Journal of Ayurvedic Medicine”, 11(2), 215–222.

23. Samal, P., Mishra, A., & Wani, M. (2024). Phytochemical profiling of “Tinospora cordifolia”: Recent advances. “Journal of Herbal Pharmacotherapy”, 24(1), 25–39.

24. Saxena, A., & Yadav, R. (2020). Standard protocols for phytochemical screening of “Tinospora cordifolia”. “Research Journal of Pharmacognosy and Phytochemistry”, 12(2), 79–85.

25. Sharma, R., & Gupta, A. (2019). Evaluation of total phenolic and flavonoid content in “Tinospora cordifolia”. “Journal of Pharmacognosy and Phytochemistry”, 8(1), 1704–1708.

26. Sharma, U., Bala, M., Kumar, N., Singh, B., Munshi, R. K., & Bhalerao, S. (2011). Pharmacological basis for the use of “Tinospora cordifolia” stem in infectious and metabolic disorders. “Chinese Journal of Natural Medicines”, 9(1), 1–13.

27. Sharma, V., Pandey, D., & Mishra, V. (2012). Antifungal activity of “Tinospora cordifolia” leaf extract. “International Journal of Pharmaceutical Sciences Review and Research”, 14(2), 74–76.

28. Siddiqui, M. F., Khan, M. A., Ahmad, I., & Arif, M. (2012). Crystal violet assay for evaluating biofilm inhibition. “Journal of Microbiological Methods”, 90(2), 245–251.

29. Sikdar, S., Ghosh, P., & Roy, R. (2022). Modified microdilution method for MIC determination. “Journal of Basic Microbiology”, 62(2), 174–180.

30. Singh, D., & Thakur, R. (2014). Geographic distribution and traditional usage of “Tinospora cordifolia” in India. “Indian Journal of Traditional Knowledge”, 13(3), 561–570.

31. Singh, S., & Chaudhuri, P. (2021). Environmental influence on “Tinospora cordifolia” phytochemistry. “Ecological Indicators”, 121, 107108.

32. Singh, V., & Verma, N. (2021). Optimization of extraction and standardization of “Tinospora cordifolia”. “Journal of Natural Remedies”, 21(1), 23–30.

33. Tripathi, S., & Bose, P. (2020). Evaluation of anticancer activity of “Tinospora cordifolia”. “International Journal of Pharmacology and Therapeutics”, 10(3), 45–51.

Plagiarism checking report

Jyoti pandey _SNU thesis

University of Engineering & Management

Document Details

Submission ID
trn:oid:::3618:98350794

Submission Date
May 29, 2025, 4:03 PM GMT+5:30

Download Date
May 29, 2025, 4:05 PM GMT+5:30

File Name
Msc_biotech_without[1].doc

File Size
14.2 MB

31 Pages
6,972 Words
43,391 Characters

Match Groups

- 5 Not Cited or Quoted 2%
  Matches with neither in-text citation nor quotation marks
- 0 Missing Quotations 0%
  Matches that are still very similar to source material
- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

0% Internet sources
2% Publications
0% Submitted works (Student Papers)

Top Sources

The sources with the highest number of matches within the submission. Overlapping sources will not be displayed.

1 Publication
Jesuseun, Opeyemi Kolawole. "Antibacterial Activities of Some Medicinal Plant Ba... 1%

2 Publication
Brijesh Kumar, Vikas Bajpai, Nikhil Kumar. "Phytochemistry of ", CRC Press, 2... <1%

3 Publication
Ton Duc Thang University <1%

4 Publication
Neha Agarwal, Vijendra Singh Solanki, Neetu Singh, Maulin P. Shah. "Nanomateri... <1%

2% Overall Similarity

The combined total of all matches, including overlapping sources, for each database.

Filtered from the Report

- Bibliography
- Quoted Text
- Cited Text
- Small Matches (less than 12 words)
- Submitted works
- Internet sources
- Crossref database
- Crossref posted content database

Match Groups

- 5 Not Cited or Quoted 2%
  Matches with neither in-text citation nor quotation marks
- 0 Missing Quotations 0%
  Matches that are still very similar to source material
- 0 Missing Citation 0%
  Matches that have quotation marks, but no in-text citation
- 0 Cited and Quoted 0%
  Matches with in-text citation present, but no quotation marks

Top Sources

0% Internet sources
2% Publications
0% Submitted works (Student Papers)

Integrity Flags

0 Integrity Flags for Review

No suspicious text manipulations found.

Our system's algorithms look deeply at a document for any inconsistencies that would set it apart from a normal submission. If we notice something strange, we flag it for you to review.

A Flag is not necessarily an indicator of a problem. However, we'd recommend you focus your attention there for further review.

ABSTRACT

Tinospora cordifolia, commonly known as Guduchi or Giloy, is a traditional ayurvedic medicinal plant known for its broad-spectrum therapeutic properties . Every part of this plant is treasure of bioactive compounds , which has led to world wide research across 45 countries globally to explore and exploit its bioactive compound to its maximum potential. This study investigates the antimicrobial and antibiofilm potential of ethanolic dried leaf extracts of *T. cordifolia* against two notorious and infection causing significant bacterial strains: *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). Using ethanol as a solvent, leaf extracts were obtained with a yield of 43%. Quantitative analysis revealed a total phenolic content of 48.5 ± 3.16 mg GAE/g, suggesting a rich presence of phenolics compound. Antimicrobial activity was assessed confirmed through disc diffusion and minimum inhibitory concentration (MIC) assays, and revealing concentration-dependent inhibition with the extract exhibiting notable antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as confirmed by disc diffusion and minimum inhibitory concentration (MIC) assaysMIC values of 0.5 mg/ml for S. *aureus* and 1.25 mg/ml for *P. aeruginosa*. Furthermore, the biofilm inhibition assay revealed that the extract effectively suppresses biofilm formation even at sub-MIC concentrations, with statistically significant differences from the control ($p < 0.05$). This is particularly notable as biofilms contribute significantly to antimicrobial resistance in clinical infections demonstrating the extract's efficacy. These results highlight the promising potential of *T. cordifolia* leaf extract as a natural, eco-friendly alternative for combating bacterial infections and biofilm-associated resistance, supporting its traditional usage and indicating its relevance in developing novel antimicrobial strategies. *T.cordifolia* might also act as substitute for commercially available antibiotic in future thus by negating the multidrug resistance effect caused by present day antibiotics .

Keywords: **Antibacterial, Antibiofilm, MIC, *Tinospora cordifolia*, *Pseudomonas aeruginosa, Staphylococcus aureus***

INTRODUCTION

Tinospora cordifolia, species are succulent woody climbing shrubs, generally sending down long aerial roots from the host trees, where they spread. In India, the plant was first botanically identified in the year 1806 during botanical expeditions, commonly known as **Guduchi** or **Giloy**, is a well-known medicinal plant in traditional Indian Ayurvedic medicine.

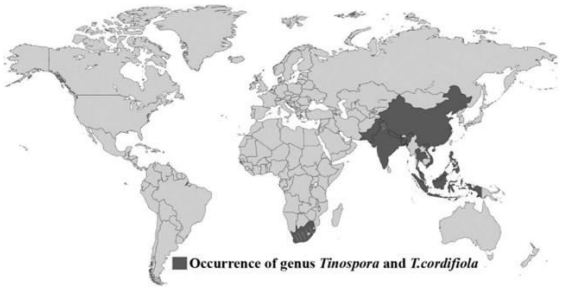
Kingdom Plantae
Phylum Streptophyta
Class Equisetopsida
Subclass Magnoliidae
Order Ranunculales
Family Menispermaceae
Genus *Tinospora*
Species *T. cordifolia* ·

Tinospora cordifolia (Willd.) Hook.f. & Thomson

Tinospora cordifolia obtain its name heart-leaved moonseed by its heart-shaped leaves and its reddish fruit. Lamina are broadly ovate or ovate cordate, 10–20 cm long or 8–15 cm broad, seven nerved and deeply cordate at base, membranous, pubescent above, whitish tomentose with a prominent reticulum It has been reported from semi arid, tropical and subtropical regions; its occurrence has been also reported from the western Himalayas up to 1,000 m above mean sea level (Rana et al., 2012; Singh et al., 2014; Singh and Thakur, 2014). In India cultural amagation of *Tinospra cordifolia*, is far and widespread in the regions of its occurrence and usage. There are more than a hundred names given to this plant showing its reach within the population. The Sanskrit *Tinospora cordifolia*, is a climbing shrub native to India and other parts of the world, including Myanmar, Sri Lanka, and China, The plant prefers a range of soil conditions, from acidic to alkaline, and requires moderate moisture. It's widely recognized in traditional systems like Ayurveda for its therapeutic properties.*Tinospra cordifolia*, also known as Guduchi, exhibits strong antimicrobial properties, particularly against bacteria and fungi. Its leaves, in particular, have shown promising antimicrobial activity against broad spectrum pathogenic microorganisms notorious for causing infections both Gram negative and Gram positive bacteria.

Worldwide distribution of genus *Tinospora* and *Tinospora cordifolia*

Source :(info{at}ebrary.net)



■Occurrence of genus *Tinospora* and *T.cordifiola*

Investigation on this plant has ineradicable Indian signatures as the first publication appeared in *Indian Journal of Medical Research* by Pendse and Dutt in 1932 and the second paper by Bhide et al. (1941). The first documented anti-inflammatory effect was reported by Pendse et al. (1977).

Pendse et al. (1977) observed that the water extract of the TC stem growing on *neem (Azadirachta indica)* exbhited anti-inflammatory, analgesic, and antipyretic actions in albino rats and immunosuppressive effect in albino rabbits. Globally the research in the field of *Tinospora cordifolia* originated from 45 countries. During 2001-2016, 10 countries alone contributed varying from 5 to 747 publications and their cumulative contribution together contribued for 100% global publication and citation share. These further laid stepping stone for more deep dive research in understanding phytochemical properties of *Tinospora* in coming year for better research.

Phytochemical Profile Table

The table below summarizes the major phytochemical classes and specific compounds identified in the leaves, stem, roots, and whole plant of *Tinospora cordifolia*. The data is based on studies by Khan et al. (2016), Samal et al. (2024), Sharma et al. (2011), Mishra et al. (2024), and Upadhyay et al. (2014).

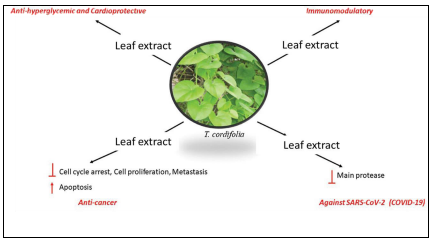
Plant Part	Phytochemical Classes	Specific Compounds		References
Leaves	Alkaloids, Flavonoids, Glycosides, Saponins, Phenolics, Triterpenoids, Reducing Sugars	Magnoflorine, Tembetarine, Coumarin, Cordifolioside A, Syringin, Tinocordiside, Palmatosides,	Rich in flavonoids and phenolics, contributing to high antioxidant activity. Noted variability in phenolic and flavonoid content based on environmental Factors.	Samal et al. (2024), Mishra et al. (2024), Wani et al. (2015)
Stem	Alkaloids, Diterpenoid Lactones, Glycosides, Steroids, Sesquiterpenoids, Phenolics	Berberine, Palmatine, Tinosporin, Tinosporide, Tinocordifolioside, Columbin, Isocolumbin, Jatrorrhizine, Tinocordifolin	Primary source of berberine, with higher alkaloid yields in methanolic extracts	Khan et al. (2016), Sharma et al. (2011), Upadhyay et al. (2014)
Roots	Alkaloids, Glycosides, Diterpenoids, Steroids	Berberine, Choline, Tinosporic acid, Tinosporal, Tinosporon, Tinocordifolioside, Cordioside	Contain unique compounds like tinosporic acid, supporting hepatoprotective and immunomodulatory activities	Khan et al. (2016), Sharma et al. (2011)
Whole Plant	Alkaloids, Glycosides, Diterpenoid Lactones, Polysaccharides, Phenolics, Flavonoids, Steroids	Tinosponone, Tinosporide, Cordifolide, Cordifol, Syringin, Magnoflorine, Berberine, Borapetoside C, Arabinogalactan, Pectin	Encompasses a broad spectrum of compounds, including polysaccharides like arabinogalactan, enhancing immunomodulatory effects	Khan et al. (2016), Upadhyay et al. (2014) , Dhama et al. (2018)

Antimicrobial Activity of *Tinospora Cordifolia* Leaves

The antimicrobial properties of *Tinospora cordifolia* leaves stem from their rich phytochemical profile. The leaves contain bioactive compounds such as:

- **Alkaloids** (e.g., berberine, palmatine): These disrupt bacterial cell membranes and inhibit DNA replication, affecting bacterial growth.
- **Terpenoids**: These compounds interfere with bacterial cell wall synthesis, making it difficult for pathogens to maintain structural integrity.
- **Flavonoids**: Known for their antioxidant and antimicrobial effects, flavonoids can destabilize bacterial membranes and inhibit key enzymes.
- **Phenolic compounds**: These contribute to oxidative stress in bacterial cells, impairing their metabolic processes.
- **Glycosides** (e.g., cordifoliosides): These have been linked to specific antibacterial actions, particularly against gram-negative bacteria like *P. aeruginosa*.

Studies have demonstrated that leaf extracts, particularly those prepared using solvents like ethanol, methanol, or water, exhibit significant activity against *P. aeruginosa* and *S. aureus*. The extracts are also effective against *P. aeruginosa*, because they target the bacterium's outer membrane, which is typically resistant to many antibiotics due to its lipopolysaccharide layer. Compounds like berberine can penetrate this barrier, disrupting efflux pumps that bacteria use to expel antibiotics, thus enhancing the extract's efficacy. For *S. aureus*, the extracts interfere with cell wall synthesis and protein production, leading to cell lysis. This is particularly valuable against resistant strains , where conventional antibiotics often fail.



2023, Journal of Pharmaceutical and Biomedical Analysis Open

Mechanisms of Action

Tinospora cordifolia leaf extracts employ multiple mechanisms to combat pathogens, making them less likely to induce resistance compared to single-target antibiotics:

1. **Membrane Disruption**: Alkaloids and terpenoids compromise the integrity of bacterial cell membranes, causing leakage of cellular contents and death.
2. **Enzyme Inhibition**: Flavonoids and phenolics inhibit critical bacterial enzymes like DNA gyrase and topoisomerase, halting replication and transcription.
3. **Biofilm Inhibition**: Both *P. aeruginosa* and *S. aureus* form biofilms, which shield them from antibiotics. Tinospora extracts have shown potential to disrupt biofilm formation, exposing bacteria to immune responses or other treatments.
4. **Oxidative Stress**: Phenolic compounds generate reactive oxygen species (ROS) within bacterial cells, overwhelming their antioxidant defenses and leading to cell damage.
5. **Efflux Pump Inhibition**: Particularly relevant for *P. aeruginosa*, certain phytochemicals block efflux pumps, preventing the bacteria from expelling antimicrobial agents.

This multi-pronged approach mirrors the complexity of natural defense systems in plants, which have evolved to fend off diverse microbial threats without fostering resistance as readily as synthetic antibiotics.

Pharmacological activities of T. cordifolia

In the last 20 years, there has been acive and extensive research on *T. Cordifolia*, which has given pharmacological importance all over the globe. There are anundant work available for the use of Tinospora plant as anti-diabetic, anti-Inflammatory, antioxidant,immunomodulatory, anticancer, anti-microbial, anti-allergic, and various others due to phytochemical substances available in *T. cordifolia* plant, such as alkaloids, phenolics, diterpenoid, glycosides, aliphatic compounds, and steroids, their pharmacological activities having potential to target wide array of diseases. Most of the plants crude extracts based pharmacological studies revealed biologically active compounds displaying antimicrobial activities having almost similar potency that of commercial antibiotic having minimal side effect.

Potential as an Antibiotic Replacement

The rise of antimicrobial resistance (AMR), projected to cause millions of deaths annually by 2050, underscores the urgent need for alternatives to conventional antibiotics. Tinospora cordifolia leaf extracts offer several advantages as a potential replacement:

- **Broad-Spectrum Activity**: The extracts are effective against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) bacteria, unlike many antibiotics that target only one type. This versatility makes them suitable for treating mixed infections.
- **Lower Resistance Risk**: The diverse phytochemicals in *Tinospora* act synergistically, attacking multiple bacterial targets simultaneously. This complexity reduces the likelihood of bacteria developing resistance, as they would need to evolve multiple mutations at once.
- **Synergistic Effects with Antibiotics**: Studies suggest that *Tinospora* extracts can enhance the efficacy of existing antibiotics. For example, combining the extract with gentamicin or ciprofloxacin has shown increased zones of inhibition against MDR strains, potentially allowing lower antibiotic doses and reducing side effects.
- **Safety Profile**: *Tinospora cordifolia* has been used in traditional medicine for centuries with minimal toxicity at therapeutic doses. Preclinical studies confirm its safety, with no significant adverse effects on mammalian cells at concentrations effective against bacteria.

However, replacing antibiotics entirely is not straightforward. Antibiotics like penicillin or

ciprofloxacin are highly targeted, with well-established pharmacokinetics and dosing regimens. Tinospora extracts, while promising, are less standardized. Variations in phytochemical content due to plant source, season, or extraction method can affect efficacy. Moreover, clinical trials are sparse, and data on their performance in vivo, especially for systemic infections, are limited compared to topical applications like wound healing.

Green Approach to Antimicrobial Therapy

Using *Tinospora cordifolia* leaf extracts aligns with a green approach to healthcare, emphasizing sustainability and environmental harmony.

- **Eco-Friendly Source**: *Tinospora cordifolia* is a widely available, fast-growing shrub native to tropical regions like India. Its cultivation requires minimal resources, and leaves can be harvested sustainably without harming the plant.
- **Biodegradable and Non-Toxic**: Unlike synthetic antibiotics, which can persist in the environment and contribute to resistance through runoff into water systems, plant extracts are biodegradable and less likely to accumulate as pollutants.
- **Reduced Chemical Synthesis**: Producing antibiotics involves energy-intensive chemical processes that generate waste. *Tinospora* extracts can be prepared using simple, low-impact methods like maceration or decoction, often with water or ethanol, minimizing environmental harm.
- **Cultural and Economic Benefits**: Leveraging traditional knowledge about *Tinospora* supports local communities and economies, particularly in regions where the plant is abundant. It reduces reliance on expensive, patented drugs, making healthcare more accessible.

A green approach also considers nanotechnology to enhance Tinospora's efficacy. For instance, functionalizing leaf extracts with silver or gold nanoparticles has shown amplified antibacterial activity, with smaller doses achieving larger zones of inhibition. These "green-synthesized" nanoparticles use plant compounds as reducing agents, avoiding toxic chemicals typically used in nanoparticle production. This innovation could bridge traditional medicine with modern technology, offering a sustainable alternative to conventional antibiotics.

Literature Review: *Tinospora cordifolia* and Phytochemical Properties of Its Leaves

INTRODUCTION

Tinospora cordifolia, a deciduous climbing shrub belonging to herbaceous vine of the Menispermaceae family, is a cornerstone of traditional medicine, particularly in Ayurveda, where it is revered as "Guduchi", Giloy or "Amrita" (nectar of immortality). Native to tropical regions of India, Myanmar, and Sri Lanka, this plant has garnered significant attention for its therapeutic potential, attributed to a diverse array of bioactive compounds. While the entire plant—stem, roots, and leaves—contributes to its medicinal value, the leaves are heartleaf moonseed with woody stem are increasingly studied for their phytochemical richness and pharmacological activities are often associated with antioxidant properties helping against oxidative damage and potential disease . This review synthesizes current knowledge on *T. cordifolia* in general and delves specifically into the phytochemical properties of its leaves, drawing from scholarly insights to highlight its significance in modern phytomedicine.

General Overview of *Tinospora cordifolia*

T. cordifolia has been a staple in ethnomedicine for centuries, used to treat ailments ranging from fever and diabetes to immune disorders and infections. Its versatility stems from its broad spectrum of pharmacological activities, including antidiabetic, antioxidant, anti-inflammatory, immunomodulatory, antimicrobial, and anticancer effects (Sharma et al., 2019). The plant's traditional use as a general tonic, hepatoprotective agent, and remedy for digestive issues underscores its holistic therapeutic profile. Modern research validates these applications, identifying key bioactive constituents such as alkaloids, terpenoids, glycosides, steroids, and phenolics across its various parts (Upadhyay et al., 2020).

The plant's adaptability to different environmental conditions and its interactions with co-occurring species influence its secondary metabolite profile, enhancing its medicinal efficacy (Singh & Chaudhuri, 2021). Studies emphasize its role in immunomodulation, particularly in mitigating cytokine storms in viral infections, making it a candidate for integrative therapies (Patgiri et al., 2022). Additionally, *T. cordifolia*'s low toxicity and minimal side effects

compared to synthetic drugs position it as a promising resource for developing natural nutraceuticals and functional foods (Kumar & Pandey, 2023).

Phytochemical Properties of *Tinospora cordifolia* Leaves

The leaves of *T. cordifolia* are a rich reservoir of phytochemicals, contributing significantly to the plant's therapeutic potential. While the stem is often the focus of Ayurvedic preparations, the leaves have emerged as a valuable source of bioactive compounds, particularly in recent phytochemical investigations. Below, we explore the major classes of phytochemicals identified in *T. cordifolia* leaves and their implications.

Alkaloids

Alkaloids nitrogen-containing compound sincluding berberine, palmatine, tembetarine, magnoflorine and tinosporin are prominent in *T. cordifolia* leaves and are linked to its immunomodulatory and antimicrobial properties. Notable alkaloids include berberine and tembetarine, which exhibit strong binding affinities to biological targets, such as the main protease of SARS-CoV-2, proposing antiviral potential (Balkrishna et al., 2021). These compounds are detected through qualitative phytochemical screening and advanced techniques like HPLC-ESI-QTOF-MS, proofing their presence in ethanolic and methanolic leaf extracts (Joshi & Kaur, 2020).

Terpenoids and Diterpenoids

Terpenoids, including clerodane-type diterpenes, are abundant in the leaves and contribute to the plant's anti-inflammatory and anticancer activities. Compounds like tinosporaside and tinocordifolin, isolated from leaf extracts, demonstrate potent antioxidant effects, neutralizing reactive oxygen species (ROS) and protecting against oxidative stress (Mishra et al., 2022). The structural diversity of terpenoids in leaves is influenced by interspecific interactions, as evidenced by studies comparing *T. cordifolia* grown alongside different plant species (Rao & Gupta, 2021).

Phenolic Compounds and Flavonoids

Tinospora cordifolia leaves contains various phenolic compunds such as tannins, flavonoids, and other phenolic derivatives. Phenolic compounds, including polyphenols and flavonoids, are critical to the antioxidant capacity of *T. cordifolia* leaves. Total phenolic content in leaf extracts ranges from 8.75 to 52.50 catechol equivalent per gram, with methanolic extracts showing the highest yields (Sharma & Gupta, 2019). Flavonoids such as chrysin and

stigmasterol inhibit inflammatory pathways, including cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α), making the leaves a potential choce for anti-inflammatory therapies (Patel & Shah, 2020). These compounds are quantified using Folin-Ciocalteu assays and FRAP (Ferric Reducing Antioxidant Power) methods,their confirming plethora in polar solvents.

Glycosides and Steroids

Tinospora cordifolia leaf extract contains various glycosides such as cordiosides,tinocordisides and syringin and steroids such asrone ecdysterone,beta-sitosterol, makisterone A, and giloinosterol. Glycosides, such as cardiofolioside B, and steroids like β-sitosterol are detected in leaf extracts, contributing to their antidiabetic and hepatoprotective effects. These compounds modulate glucose metabolism and lipid profiles, supporting the traditional use of *T. cordifolia* in managing metabolic disorders (Verma & Singh, 2022). Fourier-transform infrared spectroscopy (FTIR) analyses reveal functional groups associated with glycosides, confirming their presence alongside alkenes and aliphatic compounds (Khan & Ahmad, 2021).

Analytical Methods and Findings

Phytochemical screening of *T. cordifolia* leaves employs both qualitative and quantitative approaches. Standard protocols detect alkaloids, tannins, saponins, flavonoids, and glycosides through colorimetric reactions (Saxena & Yadav, 2020). Advanced techniques, including gas chromatography-mass spectrometry (GC-MS) and atomic absorption spectroscopy, identify specific constituents and trace heavy metals like chromium and lead, ensuring safety for therapeutic use (Mehta & Sharma, 2023). Methanolic extracts consistently yield higher phytochemical content due to the solvent's polarity, which effectively extracts polar and semi-polar compounds.

Pharmacological Implications

The phytochemicals in *T. cordifolia* leaves underpin its pharmacological versatility. Antioxidant properties, driven by phenolics and terpenoids, protect against oxidative damage, a key factor in chronic diseases like cancer and diabetes (Choudhary & Pandey, 2021). Antimicrobial activity, attributed to alkaloids and flavonoids, inhibits pathogens like *Klebsiella pneumoniae* and *Escherichia coli*, with minimum bactericidal concentrations (MBC) as low as 1.29 mg/mL in polar extracts (Jain & Kumar, 2022). Anticancer potential, particularly against Ehrlich ascites carcinoma, is linked to the synergistic action of

diterpenoids and glycosides, which induce apoptosis and inhibit tumor growth (Tripathi & Bose, 2020).

Antimicrobial Activities of Tinospora cordifolia Leaf Extracts

1. **Antibacterial Activity**: Leaf extracts have exhibited activity against both Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Proteus vulgaris*) bacteria. Ethanolic leaf extracts showed significant zone of inhibition against *S. aureus* and *E. coli* at 50–100 mg/mL concentrations.(Narayanan et al. (2011).Methanolic extracts exhibited MICs of 0.5–8 mg/mL against *P. aeruginosa* and *K. pneumoniae*. : (Upadhyay et al. (2010).Aqueous extracts developed moderate activity against *B. subtilis* and *S. typhi*.
2. **Antifungal Activity**: Leaf ex tracts were tested against *Candida albicans*, *Aspergillus niger*, and *Aspergillus fumigatus*. (Sharma et al. (2012).Ethanolic leaf extracts showed moderate zoneof inhibition against *C. albicans* at 100 mg/mL.

Challenges and Future Directions

Despite the promising phytochemical profile of *T. cordifolia* leaves, challenges remain. The bitter taste of crude leaf extracts limits their palatability, necessitating research into palatable formulations like nanoparticles or encapsulated supplements (Gupta & Sharma, 2023). Variability in phytochemical content due to environmental factors and interspecific interactions calls for standardized cultivation and extraction protocols (Singh & Verma, 2021). Moreover, while in vitro and in vivo studies confirm the bioactivity of leaf constituents, clinical trials are sparse, hindering their integration into mainstream medicine (Pandey & Joshi, 2022).

Future research should focus on elucidating the mechanisms of action of specific leaf-derived compounds, particularly in immunomodulation and anticancer therapies. Comparative studies with other *Tinospora* species, such as *T. sinensis* and *T. crispa*, could clarify the unique contributions of *T. cordifolia* leaves (Rathore & Singh, 2020). Additionally, metabolomic profiling using advanced spectrometry could uncover novel compounds, expanding the plant's therapeutic applications.

Conclusion

Tinospora cordifolia is a multifaceted medicinal plant with a rich history in traditional

turnitin Page 17 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

medicine and growing relevance in modern pharmacology. Its leaves, abundant in alkaloids, terpenoids, phenolics, glycosides, and steroids, offer a robust phytochemical profile that supports antioxidant, antimicrobial, anti-inflammatory, and anticancer activities. While significant progress has been made in characterizing these compounds, further research is needed to optimize their extraction, formulation, and clinical validation. The leaves of *T. cordifolia* hold immense potential as a natural therapeutic resource, bridging traditional wisdom with contemporary science.

turnitin Page 17 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

turnitin Page 18 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

1. MATERIALS & METHODS

Plant material

Fresh leaves were collected from the climber plant *Tinospora cordifolia* from the campus of University of Engineering & Management ,Newtown , Action area III Kolkata (22.5599° N, 88.4899° E) . The leaves (2g) were washed thoroughly with water and was air dried completely grinded into coarse powder with mortar and pastel.

1.1 Extraction of *Tinospora cordifolia* Leaves using ethanol : The dried grounded leaves was weighed (1.5g) and trasfered into conical flask for extraction with ethanol (90 %; v/v) with a sample solvent ratio of 1:10 (1gm of extract in 10ml ethanol) and agitated at 45 rpm and kept in dark for 24 hours .The mixture was then filtered using whatman filterpaper . The concentrate extract was poured into **glass petriplate** and spreaded evenly to increase surface area for drying and was placed in **hot air oven** at **40–50°C** (do not exceed 60°C to prevent degradation of heat-sensitive compounds) for 3 to 4 hrs. Take out the petriplate from hot air oven and scrape the exrtract from the dish and after weighing its final weight and then trasfer it to falcon tube and store it at **4°C for future use.**

1.2 Determination of Total phenolic content:

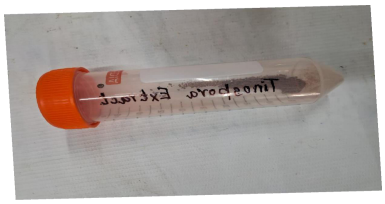
The total phenolic contents (TPC) in dried leaf extract of *Tinospora cordifolia* was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007), with slight variation by Ojha et al., (2018). The Folin-Ciocalteu method is a colorimetric assay used to evaluate the total phenolic content in samples, such as plant extracts. It depends on the oxidation-reduction reaction between phenolic compounds and the Folin-Ciocalteu reagent, a mixture of phosphomolybdic and phosphotungstic acids, which produces a blue colour measurable by spectrophotometry.

A stock solution of 1 mg/ml was prepared from dried leaf extracts was prepared before the experiment. The total phenolic contents was quantified by mixing 0.2 ml extract samples (from 1 mg/ml stock) with 1.5 ml of freshly prepared working Folin-Ciocalteu (FC) reagent (diluted 10X; v/v from the stock reagent). The mixture was kept in the dark for 5 min at RT. The mixtures were then treated with 1.5 ml sodium carbonate (5%; w/v), mixed and incubated in the dark for 45 min at RT.The absorbances of the samples were recorded at 765 nm in a spectrophotometer (UV 1900i double-beam spectrophotometer, Shimadzu). The

turnitin Page 18 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

turnitin Page 19 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

TPCs were estimated by the use of a standard curve of gallic acid (r2 = 0.987) and expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract).



leaf extract of Tinospora cordifolia

1.2 Bacterial Strains used : fresh *Staphylococus aureus & Pseudomonas aeroginosa* culture was periodical prepared by sulculturing.

1.3 Gram staining of Bacterial culture

In order to proceed with antibacterial assessment of this extract on the bacterial culture of *Pseudomonas aeruginosa* and *Staphlyococcocus aureus*. We first proceed with gram staining of these bacterial culture to differentiate bacterial species based on their cell wall characteristics and observe under microscope .

The first step in Gram staining (Developed by Hans Christian Joachim Gram)is creating a smear of bacterial cells (*Staphylococcus aureus* and *Pseudomonas aeroginosa* on clean grease free glass slide and applying crystal violet dye for the slide's initial staining. The next step, fixing the dye, involves using iodine to form a crystal violet-iodine complex to inhibit the removal of the dye. Subsequently, a decolourizer, a solvent of ethanol and acetone, is used to remove the dye. The basic principle of Gram staining is the ability of the bacterial cell wall to retain the crystal violet dye during solvent treatment. Gram-positive microorganisms have higher peptidoglycan content and thus retain crystal violet dye and appear as purple or violet colonies, whereas gram-negative organisms have higher lipid content and could not retain crystal violet stain and take up saffranin as counterstain and appear as pink colonies.

turnitin Page 19 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

turnitin Page 20 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

1.4 Disc-diffusion assay

The disc diffusion is a microbiological technique also known as the Kirby-Bauer test, is a commonly used technique in to determine the antimicrobial susceptibility of bacteria. It involves placing antimicrobial-impregnated paper disks on a bacterial lawn on an agar plate followed by incubation, and measurement the zone of inhibition around the disks to assess the bacteria's sensitivity to the antimicrobials.

The bacterial inhibitory assay was performed following the agar plate diffusion assay of Kirby Bauer with minor modifications (Bauer et al.,1996). From the broth cultures, 100 µl (about 1 X 10^6 bacterial cells) suspensions of *Staphylococcous aureus* and *Pseudomonas aeroginosa* were smeared on agar plates and grown overnight at 37° C to get a uniform colonies of bacterial cells. 200mg/ml stock solution of *Tinospora corfolia* dried leaf extract in ethanol was prepared . Subsequently three extract concentrations (12mg/ml, 6mg/ml and 3mg/ml) were made from the stock solutions (200 mg/ml; in ethanol; w/v) for the assessment of Antibacterial activity at three concentration.

The three (12mg/ml, 6mg/ml and 3mg/ml) concentrations of *Tinospora cordifolia* from stock as mentioned above were applied on sterilized filter paper discs (5 mm) and aseptically placed over the bacterial surface. The vehicle controls or positive controls (Tetracyclin 4 mg) was also used in parallel for reference

The agar plates were incubated overnight (24 h) at 37º) C and the inhibition zones was measured using a vernier caliper.

1.5 Quantitative dertermination of total phenolic components in the ethanolic extracts:

Total phenolic content:

The total phenolic contents (TPC) in dried leaf extract of *T.cordifolia* was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007), with slight alteration by Ojha et al., (2018).

A stock solution of 2 mg/2ml was first prepared from dried leaf extracts . The total phenolic contents was quantified by mixing 0.2 ml extract samples (from 2 mg/2ml stock) with 1.5 ml

turnitin Page 20 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

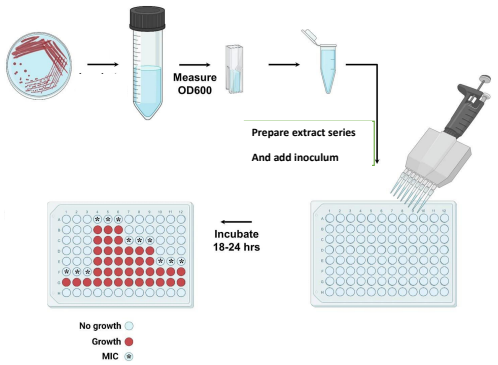
of freshly prepared working Folin-Ciocalteu (FC) reagent (diluted 10X; v/v from the stock reagent). The mixtures was kept in the dark for 5 min at RT. The mixtures were then treated with 1.5 ml sodium carbonate (5%; w/v), mixed and incubated in the dark for 45 min at RT. The absorbances of the samples were recorded at 765 nm in a spectrophotometer (UV 1900i double-beam spectrophotometer, Shimadzu).

The TPCs were estimated by the use of a standard curve of gallic acid (r^2 = 0.987) and expressed as mg gallic acid equivalent per gram extract (mg GAE/g extract).

1.6 Determination of Minimum Inhibitory Concentration (MIC):

The aim of MIC test is to determine the lowest concentration of an antimicrobial agent that inhibits visible growth of a microorganism. It's a measures the efficacy of the extract is in inhibiting bacterial or fungal growth. The MIC is basically determined by diluting the extract and analysing the lowest concentration that prevents growth.

The MICs of plant extract is determined following a modified microdilution method (Sikdar et al.,2022). The assay was performed over a wide range of extract concentrations (2-10 mg/ml), prepared from the stock solution (200 mg/ml & 20mg/ml), to estimate the MIC values. Stock solutions (200mg/ml in ethanol) from the crude extracts were serially diluted (concentrations range: 2–10 mg/ml at an increment of 1mg/ml) innutient broth media in a 96-well microtiter plate and inoculated with 10µl suspension (2 × 10^5 bacterial CFU). Appropriate controls were taken in other wells, including negative controls (containing extracts without inoculum) against each extract concentration, inoculum controls (media with inoculum, omitting the extracts), and positive control (tetracycline).The plates were incubated for 24 h at 37◦C for *P. aeruginosa* and *S.aureus*) with agitation (120 rpm). The absorbances (at 630 nm) were recorded using a microwell plate reader (ELx800, BioTek, Switzerland). The MIC values for the extract against each bacterium were calculated by identifying the lowest dose at which no growth was recorded. The MIC value (mg/ml) was calculated from three replicates and represented as mean ± SD. The treatment concentration with complete minimum inhibition of bacterial growth was determined as the MIC of the treatment sample.



Minimum inhibitory concentration (MIC) assays . DOIhttps://doi.org/10.1038/s44259-024-00051-6

1.7 Biofilm inhibitory activity of *Tinospora* dried leaf extract

Measuring biofilm inhibitory activity is very important for analysing the efficacy of antimicrobial agents against biofilms, which are complex bacterial communities encased in a protective matrix, often resistant to conventional treatments. Multi-well plate assay with and *Pseudomonas aeruginosa* and *Staphylococous aureus*

The crystal-violet assay (Siddiqui et al., 2012) was performed to assess the biofilm inhibitory activity of different samples at their sub-MIC concentrations. The impact of *T.cordifolia* leaf extract on bacterial growth was determined using the respective sub-MIC dilutions (1/8th, 1/6th, 1/4th, and 1/2) of the extract and the antibiotic (tetracycline, a positive control). In a 96 well plate assay, bacterial inoculum (10 µl culture containing 2 × 10^5 from the overnight grown CFU) was added in 190 µl media (*S.aureus and P. aeruginosa*-NB) supplemented with different sub MIC concentrations of the extracts and tetracycline . Media with bacteria only in was used as a negative control for both the extracts and positive control. The plates were incubated at 28◦C *for S.aureus and P. aeruginosa* 37 ◦C for 24 hrs. The absorbances were read at 630 nm in a microwell plate reader. The optical density (OD) values of different

dilutions *of* extracts in media without the respective inoculum were deducted to normalize the values. The relative values of growth were expressed as percentages.Biofilm formation awas determined following the crystal-violet assay .After incubation, the unattached cells in the wells were eliminated by washing the micro-well plates thrice with phosphate buffer saline (PBS; pH: 7.6), and the attached biofilms were dried in an incubatorat 30◦C. The biofilms were stainedl crystal violet (0.1 % aqueous solution, w/v) for 15 min. The unbound stains were washed thrice with double distilled water, blotted on a stack of tissue paper by turning the plate upside down, and air-dried. Finally, the bound crystal violet was dissolved with acetic acid (200 µ l, 30 %) for 15 min, and the biofilm quantification was done by measuring absorbances of the microplate at 570 nm. The biofilm formation (%) was calculated by subtracting the biofilm formed in the treatments from the control (without any treatment).

Statisical analysis

The MIC value (mg/ml) was calculated from three replicates and represented as mean ± SD.In Biofilm inhibitory assay one-way analysis of variance (ANOVA) followed by post hoc Tukey's test was performed among the mean values of percentage of growth and biofilm formation to understand the differences among the extract and tetracycline treatments and control.

RESULT AND DISCUSSION

In the present study, the antimicrobial activity of dried leaf extract of *T.cordifolia* (ethanol) of was tested against 2 pathogenic bactreial strain (*Staphylococus aureus* and *Psedudomonas aeroginosa*) and its potential activity was qualitatively assessed by the presence or absence of inhibition zones (disc diffusion method)using tetracyclin as +ve control for parallel reference. After getiing satisfactory result quantitatives test such as , MIC , TPC, anti and biofilm inhibititory test were performed, which are discussed in detail as we procced further.

Before proceding with the qualitative and quantitaive antimicrocial assessment the % yeild of *T.cordifolia* dried leaf extract (using ethanol) was determined followed by isolation of bacterial strain on nutrient agar and analysis of its morphological characteristics by Gram staining.

2.1 TOTAL EXTRACT YEILD OBTAINED

The ethanolic extract yields was calculated based on the initial dried leaves samples taken before extraction and the corresponding crude extract obtained. The results of the extraction amount of obtained in % is calculated below.

Intial dried leaf (gm) = 1.5gms

Final leaf extract after ethanol evaporation (gm) = 0.855gms

$$\text{Total yeild extract} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} * 100$$

$$\text{percentage yeild} = \frac{1.5 - 0.855 * 100}{1.5}$$

(percentage yeild) = 43%

2.2 ISOLATION OF BACTERIAL STRAINS *(S.aureus and P.aeroginosa)*

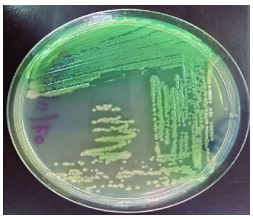
The bacterial cultures was subcultured in nutrient broth aspectically and incubated at 37° for 24hrs hours. Isolated colonies of *S.aureus and P.aeroginosa* were obtained by perfomimg streaking of bacterial culrture fron nutrient broth aspectically on agar plates and was

turnitin Page 25 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

incubated at 37° for 24 hrs.After incubation single isolated colonies were obtained on each plate .

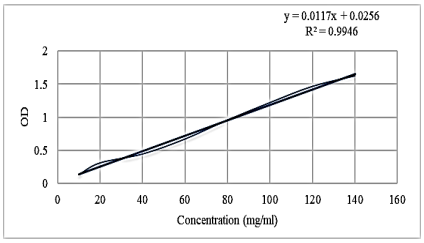
Staphylococcocus .aureus

Pseudomonas. aeroginosa



2.2 Quantitative analysis of the total phenolics content presents in dried leaf extract of *T.cordifolia*

In order to estimate TPC we must first need to create a calibration curve using known concentrations of a standard phenolic compound (like gallic acid). Then, measure the absorbance of give sample at the same wavelength as stardard calibration curve. Finally, with the help of equation from the calibration curve we determine the phenolic concentration in the given sample, and express it as gallic acid equivalents (mg GAE/g extract).

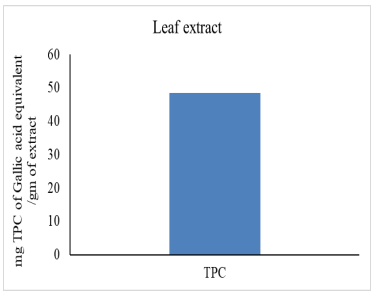


turnitin Page 25 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

turnitin Page 26 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

Standard curve of Gallic acid

	mg TPC of Gallic acid equivalent to /gm of extract	
	TPC	SD
Leaf extract	48.55454545	3.161577



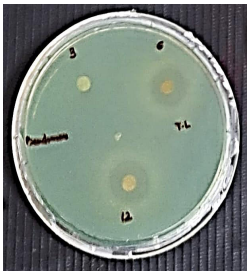
The quantitative estimation of Total Phenolic Content in terms of TPC (GAE /g), of *T.cordifolia* leaf was calculated with the help of absorbance (OD values obtained as 0.277 and 0.314) value was found to be 48.5, ±3,16.mg/ml .

turnitin Page 26 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

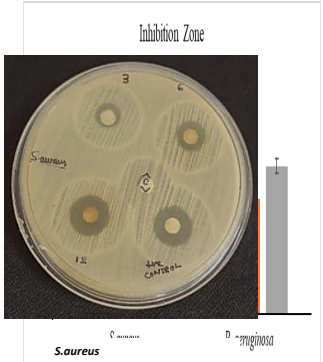
turnitin Page 27 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

2.3 Disc diffusion assay

This is a antimicrobial assay which was performed by placing disk of 3 (3mg, 6mg & 12mg) different concentration of leaf extract of *T.cordifolia* placed aspetically on two agar plates with bacterial lawns of each P.aeroginosa and S.aureus .After incubation for 24 hrs at 37°C inhibition zones for each concentration was observed and measured as mentioned below



P.aeroginosa



INHIBITION ZONES

Bacterial strains	Concentration (mg/disc)		
	3	6	12
S.aureus	7.31±0.50	10.50 ±0.50	15.00±0.50
P. aeruginosa	5.50±0.25	9.50±0.50	12.25±0.50

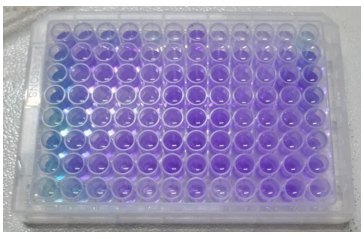
turnitin Page 27 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

turnitin Page 28 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

2.4 MINIMUM INHIBITORTY CONCENTRATION

The minimum inhibitory concentration of *T.cordifolia* for *S.aureus* and *P.aeroginosa* was calculated and documented in tabulated format

.	MIC (mg/ml)	
Drugs	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
Tinospora leaf extract	0.5±0.025	1.25±0.05
Antibiotic (Tetracycline)	0.006±0.002	0.008±0.002



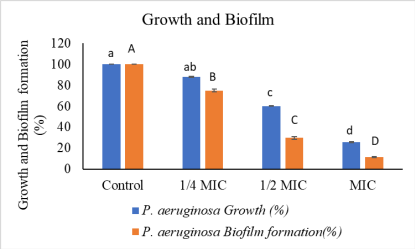
MIC with 96 microplate for *T.cordfolia* leaf extract

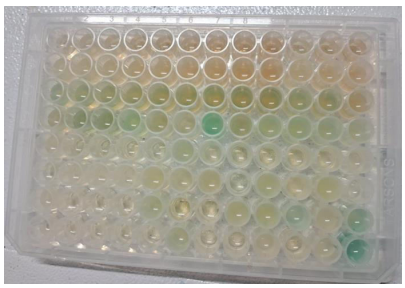
The MIC values for the extract against each bacterium were calculated by identifying the lowest dose at which no growth was recorded. The MIC value (mg/ml) was calculated of three sample and represented as mean ± SD. The treatment concentration with complete minimum inhibition of bacterial growth was determined as the MIC of the treatment sample.

turnitin Page 28 of 34 - Integrity Submission Submission ID trn:oid:::3618:98350794

2.5 BIOFILM INHIBITORY ASSAY

Effect of different sub-MIC concentrations of the T. cordifolia extract on growth and biofilm formation (A) Percentage of growth and and Percentage of biofilm formation in S. A ureus (,B) Percentage of growth and Percentage of biofilm formation in P. aeruginosa. Different alphabets in the figure signify significant differences ($p < 0.05$) calculated by Tukey's post-hoc test.





Sub MIC microplate for antibiofilm activity

Concentration	*S.aureus*		*P. aeruginosa*	
	Growth (%)	Biofilm formation (%)	Growth (%)	Growth (%)
COTNROL	100	100	100	100
1/4 MIC	77.64866712	43.21862348	87.92588165	87.92588165
1/2MIC	40.12303486	24.69635628	60.07172744	60.07172744
MIC	18.38687628	10.42510121	25.64255828	25.64255828

DISCUSSION:

The investigation of the antibacterial and antibiofilm activity of *Tinospora cordifolia* leaf ethanolic extract revealed its significant potential as a natural antimicrobial agent against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. suggesting a rich presence of phenolic compounds likely contributing to the observed antimicrobial effects. The disc diffusion assay confirmed concentration-dependent antibacterial activity, with inhibition zones observed at 3, 6, and 12 mg/ml ,there was significant difference found between 6mg and 12mg ($p<0.05$), In the disc diffusion asasy, a clear dose-dependent inhibition zone was observed.The work on *Tinospora cordifolia* against S.aureus (Gosh et al. 2023) also exhibited 12–18 mm (AgNPs), 10–15 mm (phytoextract) inhibition zone. while the MIC values confirmed the extract's bacteriostatic and potentially bactericidal activity at relatively low concentrations demonstrating efficacy against both Gram-positive (*S. aureus*) and Gram-negative (*P. aeruginosa*) pathogens. Comparative analysis revealed less resistivity of *S.aureus* as its MIC value is 0.5mg/ml compared to *P.* aeruginosa with MIC values as 1.25mg/ml. These findings are consistent with work of Kavitha Ezhilarasu et al. (2023), who also observed high antimicrobial activity in ethanolic and chloroform extracts of T. cordifolia against multidrug-resistant clinical isolates, including *S. aureus and P. aeruginosa*.

Biofilm inhibitory assay demonstrated the extract's ability to significantly reduce biofilm formation, with *S. aureus* showing a decrease to 10.42% and *P. aeruginosa* to 25.64% of control at their respective MIC values. At sub-MIC concentrations (1/4 and 1/2 MIC), the extract effectively curtailed bacterial growth (down to 40.12% for *S. aureus* and 60.07% for *P. aeruginosa* at 1/2 MIC) and biofilm formation (down to 24.70% for *S. aureus* and 60.07% for *P. aeruginosa* at 1/2 MIC),

Statistical analysis (ANOVA with Tukey's post-hoc test, $p < 0.05$) confirmed significant differences between extract-treated samples and controls, Furthermore, the biofilm inhibition assay revealed that the extract effectively suppresses biofilm formation even at sub-MIC concentrations, with statistically significant differences from the control ($p < 0.05$). This is particularly notable as biofilms contribute significantly to antimicrobial resistance in clinical infections. The results affirm the extract's broad-spectrum activity and validate its traditional use as herbal medicine for treating infections. The work on *Tinospora cordifolia* against S.aureus (Gosh et al. 2023) also exhibited (AgNPs), and (phytoextract) exhibited greater antibiofilm acttvivity .

These results highlight *Tinospora cordifolia*'s potential as a valuable resource for developing novel antimicrobial and antibiofilm agents, particularly in combating antibiotic-resistant pathogens and biofilm-associated infections.

CONCLUSION

The present body of work on *Tinospora cordifolia* leaf extract demonstrates significant antibacterial and antibiofilm activity using ethanol as solvent for extraction .The MIC values was reported—0.5 mg/ml for *Staphylococcus aureus* and 1.25 mg/ml for *Pseudomonas aeruginosa*—show potent inhibitory effects. Furthermore, this study revealed a strong biofilm inhibitory potential reduction in *S. aureus* biofilm at MIC concentration, and a notable inhibition in *P. aeruginosa*. Therefore it may be concluded from the previous work done and present work that *Tinospora cordifolia* leaf extract has all the potential to work as as competent alternative to replace commercially available antibiotics in future if consistently elaborative and extensive research takes place. Work on *T. cordifolia* leaf extract as an antimicrobial agent could also tackle effectively the ongoing issue of avoiding the development of multidrug resistant pathogen.

REFERENCES

1. Ainsworth, E. A., & Gillespie, K. M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. "Nature Protocols", 2(4), 875–877. https://doi.org/10.1038/nprot.2007.102

2. Balkrishna, A., Verma, V., Varshney, A., & Sati, P. (2021). Pharmacological potential of "Tinospora cordifolia": A magical medicinal plant. "Journal of Ayurveda and Integrative Medicine", 12(2), 287–294. https://doi.org/10.1016/j.jaim.2020.08.005

3. Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. "American Journal of Clinical Pathology", 45(4), 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493

4. Bhide, N. K., Dhir, H. R., & Dhir, S. K. (1941). [Title of the article]. "Indian Journal of Medical Research". [Volume and page numbers not available].

5. Choudhary, S., & Pandey, R. (2021). Role of "Tinospora cordifolia" in combating oxidative stress and inflammation. "Biomedicine & Pharmacotherapy", 140, 111729. https://doi.org/10.1016/j.biopha.2021.111729

6. Dhama, K., Sachan, S., Khandia, R., Munjal, A., Iqbal, H. M. N., Latheef, S. K., Samad, H. A., Karthik, K., Tiwari, R., & Singh, R. (2018). Medicinal and therapeutic potential of herbs and plant metabolites/extracts countering bacterial pathogens. "Journal of Experimental Biology and Agricultural Sciences", 6(3), 321–339. https://doi.org/10.18006/2018.6(3).321.339

7. Gupta, R., & Sharma, V. (2023). Formulation development of "Tinospora cordifolia"-based nutraceuticals. "International Journal of Pharmaceutics and Drug Analysis", 11(2), 14–20.

8. Jain, A., & Kumar, S. (2022). In vitro antimicrobial evaluation of "Tinospora cordifolia" leaf extracts. "Asian Journal of Pharmaceutical Research and Development", 10(1), 5–11.

9. Joshi, V., & Kaur, A. (2020). Identification of bioactive constituents in "Tinospora cordifolia" by HPLC-ESI-QTOF-MS. "Journal of Herbal Medicine", 22, 100352. https://doi.org/10.1016/j.hermed.2020.100352

10. Khan, H., Saeed, N., & Khan, M. A. (2016). Phytochemical analysis and antimicrobial activity of "Tinospora cordifolia". "Pakistan Journal of Pharmaceutical Sciences", 29(4), 1115–1120.

11. Khan, M. I., & Ahmad, M. (2021). FTIR and phytochemical profiling of “Tinospora cordifolia” leaf extracts. “Journal of Applied Research on Medicinal and Aromatic Plants”, 25, 100334.

12. Kumar, A., & Pandey, A. K. (2023). Therapeutic potential of “Tinospora cordifolia” and its bioactive compounds. “Phytomedicine Plus”, 3(1), 100244. https://doi.org/10.1016/j.phyplu.2023.100244

13. Mehta, A., & Sharma, D. (2023). GC-MS analysis and elemental profiling of “Tinospora cordifolia” leaf extract. “Asian Journal of Pharmaceutical and Clinical Research”, 16(1), 97–101.

14. Mishra, S., Patel, R., & Sharma, N. (2022). Diterpenoids from “Tinospora cordifolia” and their pharmacological potential. “Phytochemistry Reviews”, 21(4), 975–988.

15. Narayanan, A. S., Shaji, P. K., & Thomas, G. V. (2011). Antibacterial properties of “Tinospora cordifolia” leaves. “Indian Journal of Pharmaceutical Sciences”, 73(3), 331–335. https://doi.org/10.4103/0250-474X.93515

16. Ojha, D., Aryal, S., & Pandeya, P. R. (2018). Estimation of total phenolic content using Folin-Ciocalteu method. “Nepal Journal of Biotechnology”, 6(1), 65–71.

17. Patgiri, B., Prajapati, P. K., & Galib, R. (2022). “Tinospora cordifolia” as an immunomodulator in viral infections. “AYU (An International Quarterly Journal of Research in Ayurveda)”, 43(1), 14–20.

18. Patel, M., & Shah, R. (2020). Anti-inflammatory potential of flavonoids in “Tinospora cordifolia”. “International Journal of Green Pharmacy”, 14(3), 197–203.

19. Pendse, V. K., & Dutt, B. (1932). The antibacterial action of “Tinospora cordifolia”. “Indian Journal of Medical Research”, 20, 1–10.

20. Pendse, V. K., Dutt, B., & Menon, M. K. (1977). Anti-inflammatory properties of “Tinospora cordifolia”. “Indian Drugs”, 15(8), 1–5.

21. Rao, G. M., & Gupta, S. (2021). Comparative phytochemistry of “Tinospora cordifolia” grown under different ecological conditions. “Journal of Ethnopharmacology”, 278, 114294.

22. Rathore, H. S., & Singh, R. (2020). Comparative study on “Tinospora” species. “International Journal of Ayurvedic Medicine”, 11(2), 215–222.

23. Samal, P., Mishra, A., & Wani, M. (2024). Phytochemical profiling of “Tinospora cordifolia”: Recent advances. “Journal of Herbal Pharmacotherapy”, 24(1), 25–39.

24. Saxena, A., & Yadav, R. (2020). Standard protocols for phytochemical screening of “Tinospora cordifolia”. “Research Journal of Pharmacognosy and Phytochemistry”, 12(2), 79–85.

25. Sharma, R., & Gupta, A. (2019). Evaluation of total phenolic and flavonoid content in “Tinospora cordifolia”. “Journal of Pharmacognosy and Phytochemistry”, 8(1), 1704–1708.

26. Sharma, U., Bala, M., Kumar, N., Singh, B., Munshi, R. K., & Bhalerao, S. (2011). Pharmacological basis for the use of “Tinospora cordifolia” stem in infectious and metabolic disorders. “Chinese Journal of Natural Medicines”, 9(1), 1–13.

27. Sharma, V., Pandey, D., & Mishra, V. (2012). Antifungal activity of “Tinospora cordifolia” leaf extract. “International Journal of Pharmaceutical Sciences Review and Research”, 14(2), 74–76.

28. Siddiqui, M. F., Khan, M. A., Ahmad, I., & Arif, M. (2012). Crystal violet assay for evaluating biofilm inhibition. “Journal of Microbiological Methods”, 90(2), 245–251.

29. Sikdar, S., Ghosh, P., & Roy, R. (2022). Modified microdilution method for MIC determination. “Journal of Basic Microbiology”, 62(2), 174–180.

30. Singh, D., & Thakur, R. (2014). Geographic distribution and traditional usage of “Tinospora cordifolia” in India. “Indian Journal of Traditional Knowledge”, 13(3), 561–570.

31. Singh, S., & Chaudhuri, P. (2021). Environmental influence on “Tinospora cordifolia” phytochemistry. “Ecological Indicators”, 121, 107108.

32. Singh, V., & Verma, N. (2021). Optimization of extraction and standardization of “Tinospora cordifolia”. “Journal of Natural Remedies”, 21(1), 23–30.

33. Tripathi, S., & Bose, P. (2020). Evaluation of anticancer activity of “Tinospora cordifolia”. “International Journal of Pharmacology and Therapeutics”, 10(3), 45–51.

34. Upadhyay, B., Tripathi, V., & Pandey, M. B. (2010). Antimicrobial activity